A STUDY ON LIPOPROTEIN (a) LEVELS IN YOUNG CORONARY ARTERY DISEASE PATIENTS AND THEIR FIRST DEGREE RELATIVES

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BONAFIDE CERTIFICATE

This is to certify that this dissertation work entitled **"A STUDY ON LIPOPROTEIN (a) LEVELS IN YOUNG CORONARY ARTERY DISEASE PATIENTS AND THEIR FIRST DEGREE RELATIVES"** is the original bonafide work done by **DR.A.K.ROOPA**, Post Graduate Student, Institute of Biochemistry, Madras Medical College, Chennai under our direct supervision and guidance.

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ABBREVIATIONS

1.	CAD	-	Coronary Artery Disease
2.	CHD	-	Coronary Heart Disease
3.	IHD	-	Ischemic Heart Disease
4.	MI	-	Myocardial Infarction
5.	ECM	-	ExtraCellular Matrix
6.	CADI	-	Coronary Artery Disease among Asian Indians
7.	NO	-	Nitric Oxide
8.	eNOS	-	endothelial Nitric Oxide Synthase
9.	TNF	-	Tumour Necrosis Factor
10.	ICAM 1	-	Intercellular Adhesion Molecule 1
11.	VCAM 1	-	Vascular Cell Adhesion Molecule 1
12.	PECAM 1	-	Platelet Endothelial Cell Adhesion Molecule 1
13.	LDL	-	Low Density Lipoprotein
14.	HDL	-	High Density Lipoprotein
15.	VLDL	-	Very Low Density Lipoprotein
16.	LCAT	-	Lecithin : Cholesterol Acyl Transferase
17.	CETP	-	Cholesterol Ester Transfer Protein
18.	SR-B1	-	Scavenger Receptor- B1
19.	MCP-1	-	Monocyte Chemoattractant Protein-1
20.	Hs CRP	-	High sensitivity C- Reactive Protein
21.	PAI-1	-	Plasminogen Activator Inhibitor 1
22.	TFPI	-	Tissue Factor Pathway Inhibitor
23.	NEFA	-	Non Esterified Fatty Acids
24.	ROS	-	Reactive Oxygen Species

25.	PON1	-	Paraoxonase 1
26.	Lp(a)	-	Lipoprotein(a)
27.	Apo(a)	-	Apolipoprotein(a)
28.	OxPLs	-	Oxidised phospholipids
29.	Lp- PLA_2	-	Lipoprotein associated Phospholipase A_2
30.	PAPP-A	-	Pregnancy Associated Plasma Protein A
31.	FXR	-	Farnesoid X receptor
32.	FGF19	-	Fibroblast Growth Factor 19
33.	HNF3A(FOXA1)	-	Hepatocyte Nuclear Factor 3 alpha (Forkhead Box Protein A1)
34.	HNF1A	-	Hepatocyte Nuclear Factor1 Homebox A
35.	PCSK9	-	Proprotein Convertase Subtilisin / Kexin Type 9 Serine Protease
36.	BiP	-	Endoplasmic molecular chaperone
37.	PDI	-	Protein Disulfide Isomerase.
38.	MMP-12	-	Matrix Metallo Proteinase
39.	DANCE	-	Developing Arteries and Neural Crest EGF-like
40.	EC	-	Endothelial cell
41.	PUFA	-	Poly Unsaturated Fatty Acids.
42.	COX-1	-	Cycloxygenase-1
43.	SVD	-	Single Vessel Disease
44.	DVD	-	Double Vessel Disease
45.	TVD	-	Triple Vessel Disease
46.	IFCC	-	International Federation of Clinical Chemistry
47.	EUROASPIRE	-	European Action on Secondary Prevention by Intervention to Reduce Events

Introduction

INTRODUCTION

Coronary artery disease (CAD) is the principal cause of mortality and morbidity in the developed countries. However recent evidences show that there is an alarming increase in the prevalence of coronary artery disease in South Asians.[1] Despite having lower body mass index and lower waist circumference, Coronary artery disease presents at a very young age in this population and the presentation is more severe than other population.[2] Traditional risk factors like smoking, Hypertension, Diabetes mellitus and Obesity do not completely explain the increased prevalence of CAD in younger age group. Therefore non conventional risk factors such as elevated lipoprotein(a) , homocystine, thrombogenic factors like plasminogen activator inhibitor, fibrinogen and high sensitivity CRP (hs-CRP) have gained importance recently.[3]

EPIDEMIOLOGY:

Ischemic heart disease (IHD) is a life threatening serious illness adding higher financial constraints to the patient and his/her family compared to other illness. In the United states, 13 million people suffer IHD out of which 6 million people develop angina pectoris and 7 million sustain myocardial infarction(MI).[4] Recently with the ongoing urbanisation in the developing countries, population that is commonly affected by IHD are south Asians- Indians and the people in the Middle east.[4] People of this ethnic group develop MI at a younger age of less than 40 years.[5,6] It was observed that young Asian Indians had 15 fold increased incidence of CAD compared to Chinese and 10 fold increase compared to Malays.[7] In a study conducted in UK, first incident of MI (at < 40 years) occurred

5 years earlier with a 10 fold higher rates in Indians than in Caucasians.[8] There were also higher frequency of triple vessel disease, massive infarct and ventricular dysfunction which resulted in higher rates of mortality in young Asian Indians.[9]

Elevated levels of lipoprotein(a) has been found to be an inherited independent risk factor for premature CAD in the western countries.[10,11] Enas et al first reported elevated lipoprotein(a) levels in CADI (Coronary Artery Disease among Asian Indians) study.[12] Fewer case control studies conducted in Indian population reveal lipoprotein(a) as a risk factor for the occurrence of CAD in patients below 40 years of age.[13,14] Blacks have the highest Lp(a) levels followed by Asian Indians and whites. However Blacks have less dangerous larger isoforms of Lp(a) accounting for the lower prevalence of CAD compared to Asian Indians.[15,16]

Review of literature

REVIEW OF LITERATURE

Ischemic heart disease is defined as impaired function of myocardium due to the imbalance between the blood supply and the heart's demand for oxygenated blood. The cause for myocardial ischemia is the reduced coronary blood flow due to the obstruction in the coronary arteries because of progressive atherosclerosis. Therefore ischemic heart disease is termed as coronary artery disease (CAD) or coronary heart disease (CHD).

ATHEROSCLEROSIS:

Atherosclerosis is a chronic inflammatory response characterised by lesions in the intimal layer of blood vessels. This can protrude into the vascular lumen thereby obstructing it and also causes weakening of the underlying media which may end up with serious complications. It is also referred as "hardening" or "furring" of the arteries.

NATURAL HISTORY OF ATHEROSCLEROSIS:

Pre-clinical phase (usually at young age)

1. Fatty streaks
2. Fibrofatty plaque
3. Advanced/ Vulnerable plaque

Clinical phase (usually middle age to elderly)

1. Occlusion by thrombus
2. Critical stenosis
3. Aneurysm and rupture

FATTY STREAKS:

They are multiple yellowish spots that coalesce later into elongated streaks.They are mainly formed by foam cells (cholesterol laden macrophages and smooth muscle cells) and do not cause clinically significant obstruction of the arteries. Irrespective of the age, race, environment, fatty streaks are found in children more than 10 years.[17,18] Though they are the precursor lesion for atherosclerotic plaques, development into an advanced lesion is dependant on other factors.

FIBROFATTY PLAQUE

They are distributed more commonly (in the descending order) lower abdominal aorta, coronary arteries, popliteal arteries, internal carotid arteries, and the circle of Willis. Components of fibroplaque includes the following:

1. Smooth muscle cells, macrophages and T-lymphocytes
2. Extracellular matrix (ECM)- collagen, proteoglycans and elastic fibers
3. Intracellular and extracellular lipid

A superficial fibrous cap is made up of smooth muscle cells with dense extracellular matrix.

ADVANCED/VULNERABLE PLAQUE

The plaque progressively enlarges in size due to accumulation of necrotic debris, synthesis and remodelling of ECM. The macrophages releases proteolytic enzymes like matrix metalloproteinases,[19] which digests the collagen at the

fibrous cap especially occurring at its shoulder where the cap appears thin and where the macrophages are highly concentrated. These lesions can lead to the following complications:

1. Focal rupture followed by superimposed thrombosis
2. Haemorrhage into a plaque
3. Aneurysmal dilation due to the medial atrophy

Plaques on disruption can lead to exposure of thrombogenic substances which leads to the formation of thrombus.[20] This compromises the flow of blood to the distal organs causing ischemic injury (infarction) of the tissues fed by the artery.

Symptomatically, if this happens in

1. Coronary artery – blood supply to myocardium is withheld – causes myocardial infarction
2. Carotid artery – inadequate blood supply to brain- causes transient ischemic attacks and stroke.
3. Insufficient blood supply to lower limb – causes claudication pain - peripheral artery disease and gangrene of the legs
4. Similar events are also noticed in arteries of kidney and intestines, since atherosclerosis involves most of the large and medium sized arteries.

“RESPONSE TO INJURY” HYPOTHESIS:

This hypothesis in the development of atheroma is due to the chronic inflammatory response due to the endothelial injury. Atherosclerosis begins with morphologically intact but functionally altered endothelium which permits the passage of plasma lipoproteins and inflammatory cells into the arterial wall. Normally endothelium is not thrombogenic and it is because of the glycoproteins and proteoglycans over endothelial surface, prostacyclin and nitric oxide (NO). Prostacyclin and NO are potent vasodilators and inhibitors of platelet aggregation. Stress over endothelium accompanied by turbulent blood flow, at the branch points of the vessels are more prone to develop the lesions. These haemodynamic alterations and hyperlipdemia are the two major determinants of endothelial injury. Apart from that smoking, homocysteine, viruses, inflammatory cytokines like Tumour necrosis factor (TNF) are also the potential culprits. Thus atherosclerosis occurs in a “**Vulnerable vessel in vulnerable blood**” .

Role of inflammation:

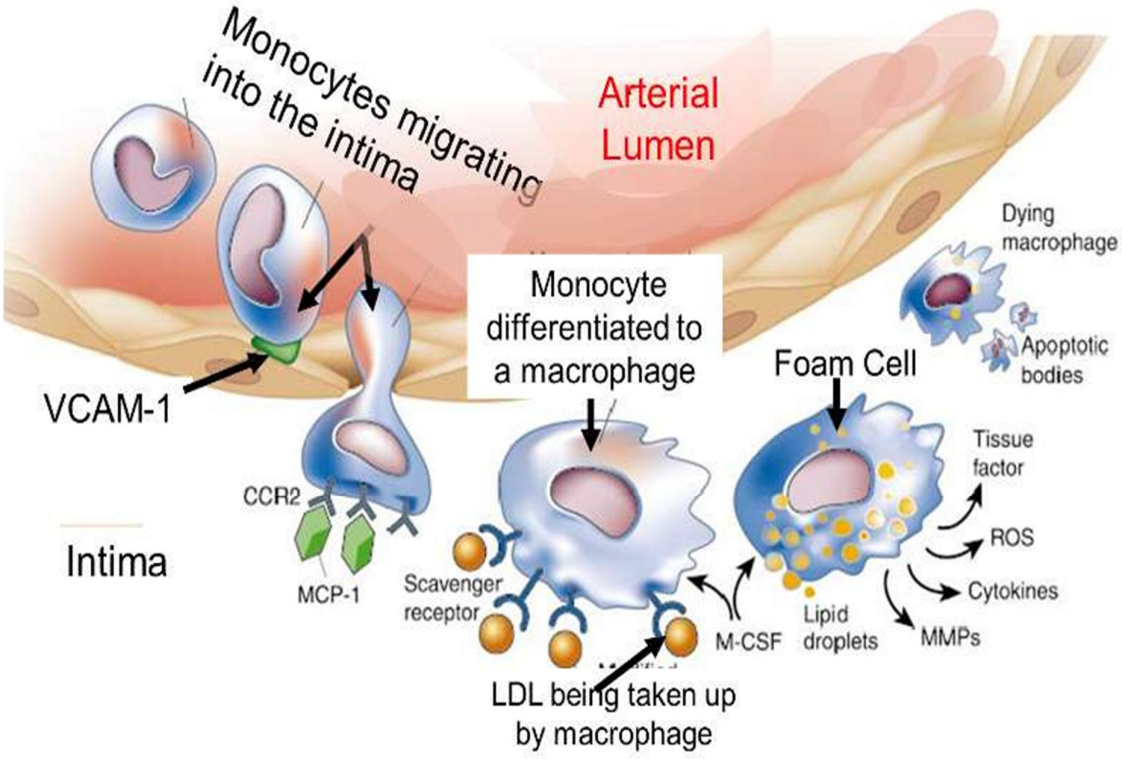
Inflammatory mechanism plays a significant role in initiation, progression till the development of complications of atherosclerosis.[21,22] Early atherogenesis involves overexpression by endothelial cells, various cell adhesion molecules like E selectin and P selectin which help in the rolling of leukocytes over endothelial surface , ICAM 1 and VCAM 1 needed for adhesion of leukocytes, PECAM/CD31 which facilitates leukocytes to transmigrate in between the endothelial cells (diapedesis)[23]

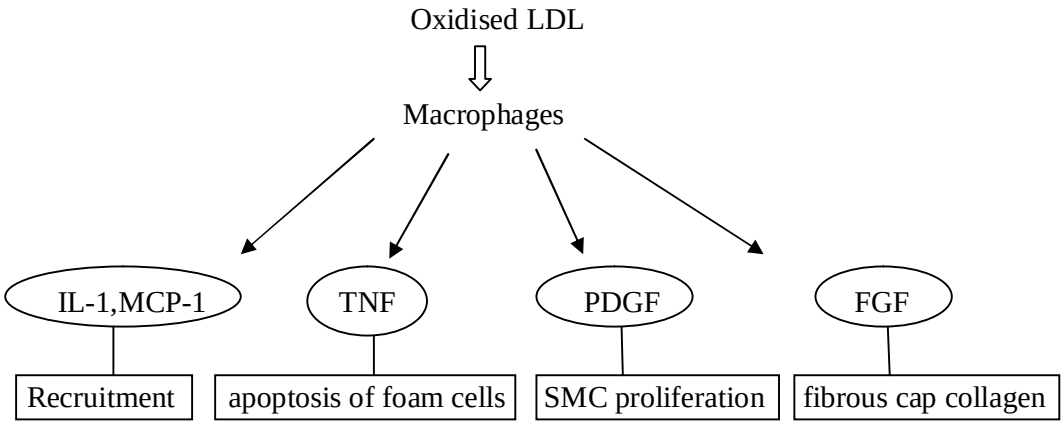
Role of lipids:

The polyunsaturated fatty acids present in LDL particle can undergo peroxidation of double bonds by the free radicals that are being generated by macrophages and endothelial cell in the artery. This leads to generation of malondialdehyde and 4- hydroxynonenal which bind to apo B-100 of LDL rendering it a net negative charge thereby making it unsuitable for recognition by native LDL receptors. In this scenario, monocytes that has been activated to tissue macrophage express SR-B1 (Scavenger receptor) which causes rapid uptake of the oxidised LDL forming foam cells.

Oxidised LDL itself is immunogenic and acts as a chemoattractant. Circulating monocytes and T lymphocytes are directed to the site and it helps in the differentiation of monocyte into tissue macrophages. Oxidised LDL will also activate macrophages to produce various cytokines like IL-1 and TNF and chemokines including MCP-1 thus increasing recruitment. The recruited T cell elaborates IFN- γ and IL-6 which in turn stimulates macrophages, endothelial cells and smooth muscle cells. Under the influence of these cytokines macrophage produce growth factors like PDGF and FGF. PDGF causes smooth muscle cell migration and proliferation. FGF stimulates the smooth muscle cell to synthesise extracellular matrix including collagen that forms the fibrous cap. TNF will induce apoptosis of foam cells liberating its lipid content thereby forming the lipid core.

Fig 1. Pathogenesis of atherosclerosis





CORONARY ARTERY DISEASE

Blood supply to myocardium occurs during diastole. There are three resistance vessels which contributes to 75% of the resistance to the coronary blood flow:

1. Large epicardial arteries (R1)
2. Prearteriolar vessels (R2)
3. Intramyocardial capillaries (R3)

R2 and R3 are the major determinants of coronary resistance.

Regulation of blood supply to myocardium :

1. Metabolic regulation**:** normally R2 and R3 (resistance) will decrease thereby allowing more blood flow through coronaries. Whenever heart demands more oxygen and nutrition like during exercise this mechanism is augmented.
2. Autoregulation: the coronary blood flow is maintained throughout irrespective of change in blood pressure.

In atherosclerosis with progressing stenosis of the vessel, the resistance vessels get dilated maximally ,but the pressure needed for myocardial blood flow decreases at the same time that leads to manifestation like angina. If the stenosis is about 50% there is limited compensation to increase blood flow whenever myocardium demands, while if the stenosis is about 80% blood flow even at rest may be reduced. Reversible damage occurs if the occlusion persists for <20 minutes and it becomes permanent if it continues > 20 minutes.

BLOOD SUPPLY OF HEART:

Blood supply	**Branches**	**Areas supplied**
Right coronary artery	Posterior interventricular artery	Right atrium,Right and left ventricle including its inferior wall, posterior part of interventricular septum
	Right marginal artery	Anterior surface of right ventricle
Left coronary artery	Circumflex artery	Left ventricle, Left atrium
	Anterior interventricular artery	Right and left ventricle, anterior part of interventricular septum

Left main coronary artery and proximal left anterior decending coronary artery are particularly hazardous. Ischemia progresses from endocardium towards epicardium.[24]

EFFECTS OF ISCHEMIA:

MECHANICAL EFFECTS: non uniform pattern of ischemia causing ventricular segmental hypokinesia, akinesia and dyskinesia reducing myocardial pumping ability

BIOCHEMICAL EFFECTS: Due to oxygen depletion, fatty acids are not oxidised. Glucose undergoes anaerobic glycolysis to form lactate. Lactic acidosis occurs and pH is reduced. ATP depletion impairs Na^+ – K^+ pump causes potassium leak and sodium uptake by myocytes.

ECG CHANGES:

1) inverted T waves- non transmural intramyocardial ischemia
2) ST segment depression- patchy subendocardial ischemia
3) ST segment elevation – severe transmural ischemia.

ELECTRICAL EFFECTS:

Ventricular premature beats, ventricular tachycardia, ventricular fibrillation

CLASSIFICATION OF CAD

Stable angina**:** Attacks occur during exercise, emotion, eating and coitus and subsides during rest. It occurs due to the fixed coronary obstruction.

Unstable angina: unpredicted attacks with progressive increase in severity when compared to the previous attack. It occurs due to the rupture of plaques. There is no myocardial necrosis.

Prinzmetal angina: unstable angina associated with coronary vasospasm.

Acute coronary syndrome:

	Unstable angina	**NSTEMI**	**STEMI**
Ischemic symptoms	+	+	+
Cardiac biomarkers	-	+	+
ST elevation	-	-	+

Criteria for the definition of acute myocardial infarction: [25]

Detection of rise of cardiac biomarkers with at least one of the following:

a. Ischemic symptoms
b. ECG changes of new ischemia [new ST-T changes or new left bundle branch block(LBBB)]
c. Development of pathological Q waves
d. Imaging evidence of new regional wall motion abnormality

RISK FACTORS FOR CORONARY ARTERY DISEASE:

CONVENTIONAL RISK FACTORS:

1. Cigarette smoking
2. Hypertension
3. Abnormal lipid profile
    a) High total and LDL cholesterol
    b) Low HDL cholesterol
    c) High triglycerides
4. Metabolic syndrome, Insulin resistance and Diabetes mellitus

NON CONVENTIONAL RISK FACTORS:

1. hs CRP
2. Other markers of inflammation like IL-1,IL-6,p- Selectin, sCD40 ligand,etc
3. Homocysteine
4. Genetic markers (9p21 risk allele and PCSK9 locus)
5. Environmental exposures:
    - Depression and mental stress
    - Physical inactivity
    - Obesity
    - Diet
    - Alcohol consumption
    - Menopause
6. Lipoprotein(a)

CONVENTIONAL RISK FACTORS:

1. Cigarette smoking:

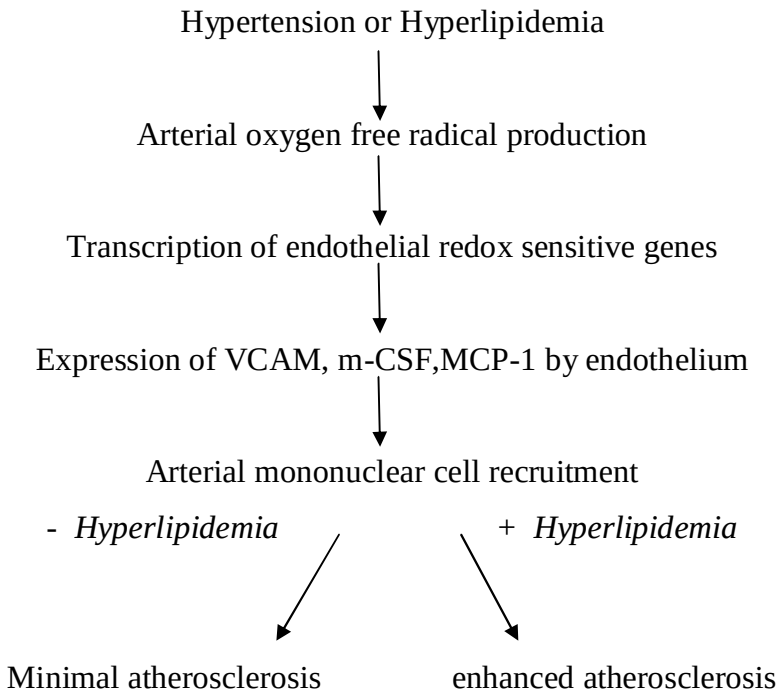
It is a dose dependant risk factor i.e., it depends on how many cigarettes a person smokes a day and at what age he/she started smoking. It increases the risk by two to three fold when coexists with other risk factors. There are many studies that describe the mechanisms by which smoking increases CAD risk. Smoking increases the levels of oxidised LDL, decreases cardioprotective High density lipoprotein (HDL) levels and increases CRP. Smoking is also found to be associated with increased fibrinogen [26] and increased platelet adhesiveness.[27]

Nicotine of cigarette enhance lipolyis thereby increasing acetyl coA pool in liver favouring cholesterol synthesis. Direct effects of carbon monoxide and nicotine will cause endothelial dysfunction and transient constriction of coronaries.

2. Hypertension:

Atherosclerosis develops in the parts of the vasculature which exerts higher pressure . Even at lower systolic pressure of 115 mm Hg and diastolic blood pressure of 75 mm Hg, risk for coronary artery disease has been observed in epidemiological studies.[28,29] The risk increases when it is associated with other risk factors like insulin resistance, dyslipidemia, obesity,etc and in only 20% of individuals it was the sole risk factor.[30]

The main pathogenic event in the development of atherosclerosis due to hypertension is the production of oxygen free radicals which stimulates NFκB pathway. NFκB is the transcription factor which enters into nucleus and promotes the transcription of adhesion molecules VCAM-1 and smooth muscle growth factors thereby promoting atherosclerosis. Haemodynamic stress due to increased pressure will also trigger plaque rupture.



3. Dyslipidemia

LDL is considered as 'bad' cholesterol and HDL is said to be 'good' cholesterol. Abnormal lipid profile resulting from the change in the ratio of LDL and HDL cholesterol levels can predispose to coronary events.

Low HDL :

HDL is an antiatherogenic lipoprotein synthesized and secreted from liver and intestine in a nascent discoidal form with phospholipid, cholesterol and apoAI on its surface. Apo AI activates the enzyme Lecithin : cholesterol acyl transferase (LCAT) where Cholesterol gets converted to cholesterol esters which move into the hydrophobic interior of the particle forming smaller and spherical HDL3.

Reverse cholesterol transport:

Scavenger receptor B1 (SR-B1) has a dual role in HDL metabolism.

1) It mediates the efflux of cholesterol from tissues to HDL,where cholesterol is esterified by LCAT to form larger lipid enriched HDL2.
2) In the liver and steroidogenic tissues, SR-B1 is the receptor for apoAI of HDL where cholesterol esters are taken up for the excretion via bile (as cholesterol or as bile acids) and for the synthesis of steroid hormones respectively.

The incidence of atherosclerosis decreases if HDL 2 subfraction is relatively high because it reflects the reverse cholesterol transport. Apart from reverse cholesterol transport, HDL exihibits antiatherogenic properties by inhibition of LDL oxidation by HDL bound paraoxonase 1 (PON1), [31,32] inhibition of platelet activation and monocyte chemoattraction and adhesion to vascular endothelium.[33]

High LDL:

LDL is an atherogenic lipoprotein formed via VLDL-IDL-LDL cascade. It is a cholesterol ester rich spherical particle with a monolayer of phospholipid, cholesterol and apo B-100 on its surface. 75% of LDL is taken up by the liver through LDL receptors while the rest is utilised by extrahepatic tissue. Uptake of LDL through LDL receptor is a regulated process ,whereas scavenger receptors present in the macrophages of subendothelial space ingest LDL in a unregulated fashion which complicates the fatty streaks.[34]

Oxidised LDL:

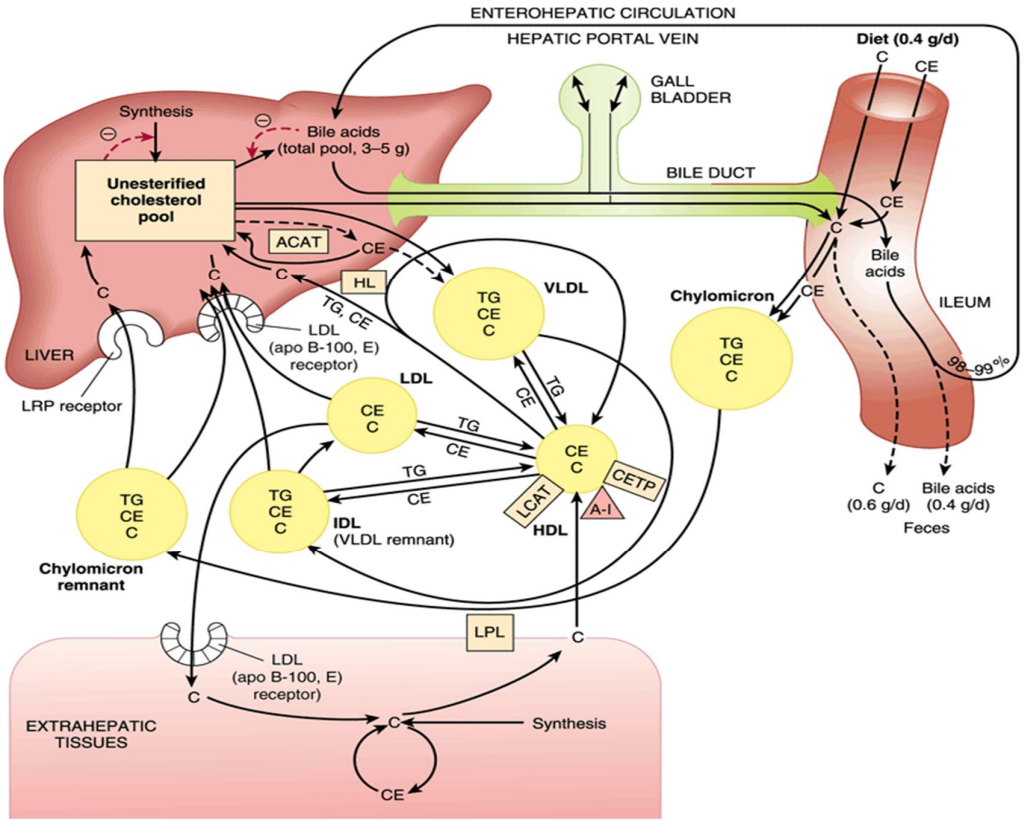
The LDL particles present in the subendothelial space [35] undergoes oxidation and is converted to oxidised LDL.[36] LDL has a high content of poly unsaturated fatty acids (PUFA) – linoleic acid and arachidonic acid [37] along with lipid soluble antioxidants like $\propto -$ tocopherol , ubiquinol-10, $\beta -$carotene and retinol.[38] The cells involved in the process of atherosclerosis, principally macrophages initiate free radical driven oxidation of PUFAs in LDL. Hydrogen atom is removed from methylene (CH2) group making the carbon unstable where molecular rearrangements take place to form conjugated diene as an intermediate. Conjugated diene reacts with molecular oxygen actively to form PUFA peroxyl radical which abstracts a hydrogen atom from the nearer PUFA forming hydroperoxide (LOOH). Lipid hydroperoxides decomposes to malondialdehyde and 4- hydroxynonenal. These reactive aldehydes binds positively charged ε amino group of lysine residues of apo B100 rendering them negative charge making it unsuitable for normal LDL receptor recognition ,in contrast increasing its affinity to scavenger receptors.[39]

Atherogenicity of oxidised LDL is because

1. Oxidised LDL is readily taken up by macrophages to form foam cells
2. It stimulates endothelium to secrete MCP-1
3. It decreases the production of nitric oxide (NO) by endothelium
4. Oxidised LDL itself is immunogenic
5. Oxidised cholesterol (oxysterol) and oxidised phospholipids are proatherogenic.[40,41]

PUFA content, available antioxidants, LDL particle size and the surrounding microenvironment (HDL and its enzymes, pH, local antioxidants) will determine the resistance to LDL oxidation.

Transport of Cholesterol between tissues



Courtesy: Harper's Illustrated Biochemistry.

Small dense LDL:

Small dense LDL is formed from VLDL where LDL exchanges its cholesterol esters to VLDL and in turn receives triglycerides aided by CETP (Cholesterol ester transfer protein). Triglyceride rich LDL is acted upon by hepatic lipase which hydrolyses triglycerides and phospholipids to form small dense LDL.

Atherogenicity of small dense LDL:

1. Because of its smaller size it can easily penetrate between endothelial cells into subintimal space.[42]
2. Small dense LDL binds with less affinity to LDL receptors so its half life in circulation is increased.[43]
3. Decreased phospholipid and cholesterol content induces conformational change in apo B 100 protein . This exposes proteoglycan binding site so that the macrophages can now easily trap apo B100 - proteoglycan complex.[44]
4. Due to its minimal antioxidant content and higher PUFAs compared to highly buoyant LDL it is subject to earlier oxidation. [45]
5. It promotes thromboxane synthesis thereby platelet aggregation.[46]

Thus small dense LDL particle number is found to increase the risk for CAD by two to three folds.[47]

Hypertriglyceridemia:

Hypertriglyceridemia directly and indirectly has been found to be the risk factor for CAD. Several mechanisms have been proposed.

1. Hypertriglyceridemia is associated with predominance of small dense LDL particles and low HDL cholesterol.
2. Several cohort studies show it is the postprandial increase in triglyceride levels that is associated with vascular events where triglyceride rich lipoprotein remnants are taken up by endothelial cells.[48]

3. Studies show that serum triglyceride levels correlates positively with clotting factors like fibrinogen and factor VII.[49]

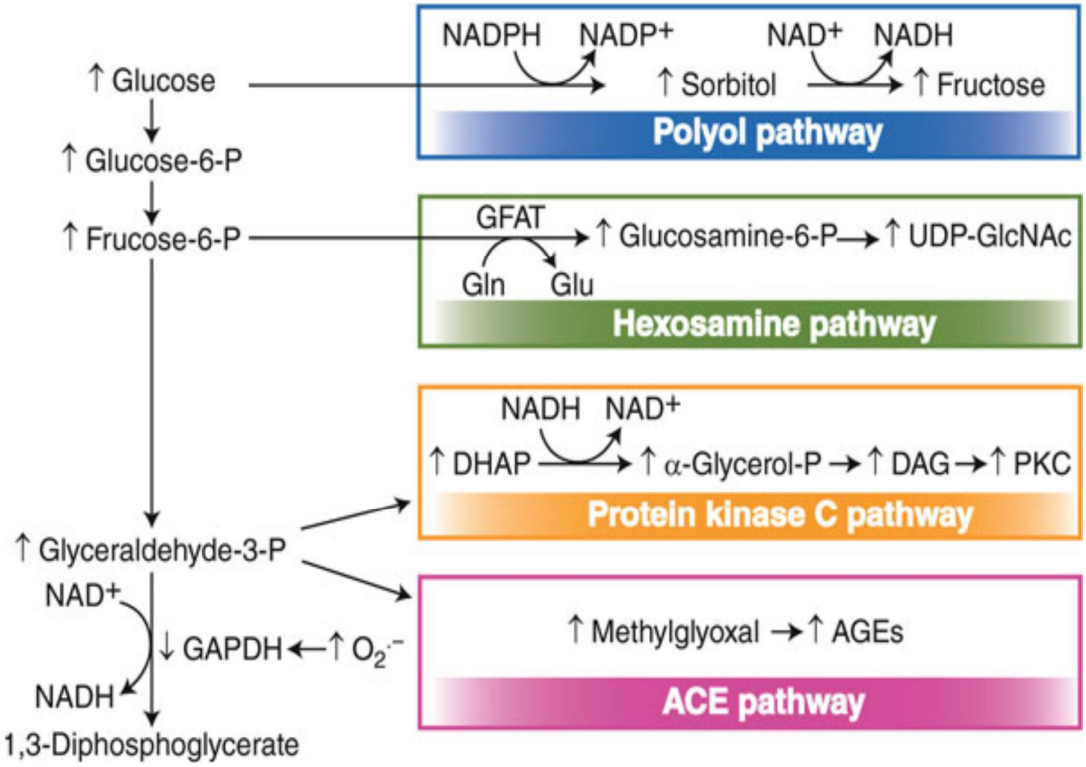
Diabetes mellitus:

Hyperglycemia increases the risk factor for CAD two to four fold in both men and women.[50,51] CHD is the primary cause of death in Diabetic patients and nearly 25% of MI patients who survived had Diabetes mellitus. Premenopausal Diabetic women and non Diabetic men both have the similar risk for CAD , hence premenopausal state will no longer serve as a protective factor for women with Diabetes.[52] Diabetics with MI are prone for multivessel disease and the percentage of stenosis is also proportional to the duration of Diabetes.[53]

The mechanism by which hyperglycemia causes atherosclerosis is through the potential production of reactive oxygen species. Increased rate of glycolysis in the setting of hyperglycemia causes increased NADH/NAD ratio, where increased flux of electrons in electron transport chain causes inhibition at complex III generating free radical intermediates of CoQ (ubiquinone). Reactive oxygen species(ROS) inhibits glyceraldehyde-3-phosphate dehydrogenase enzyme of glycolysis causing accumulation of glyceraldehyde - 3- phosphate and fructose-6-phosphate. The former forms diacylglycerol (DAG) activating protein kinase C pathway which stimulates production of proatherogenic factors and inhibit eNOS. Fructose-6- phosphate enters hexosamine pathway forming UDP N-acetyl glucosamine responsible for N-glycosylation of eNOS and its inactivation. It also results in glycosylation of transcription factor SP1 resulting in

increased expression of TGF- β and PAI-1. Furthermore increased production of methyl glyoxal, advanced glycation end product which is responsible for nonenzymatic modification of extracellular proteins, binds to RAGE (receptor for AGE) stimulating NFκB pathway responsible for proinflammatory and procoagulable state.

Pathways showing hyperglycemia induced injury



Courtesy: Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414(6865):813–20. With permission from Nature Publishing Group

Metabolic syndrome & Insulin resistance:

Insulin resistance and hyperinsulinemia have been found as an independent risk factor for CHD in non diabetic men.[54] Insulin resistance is associated with increased lipolysis in adipocytes with the release of non esterified fatty acids (NEFA). NEFA competes with glucose for substrate oxidation increasing the intracellular content of metabolites like diacylglycerol which activates the phosphorylation of Insulin receptor substrates 1 & 2. This reduces the ability of insulin receptors to undergo tyrosine phosphorylation shutting the (phosphotidyl inositol-3) PI3 cascade and stimulating the alternative MAP kinase (Mitogen Activated Protein kinase) pathway diverting from insulin's antiatherogenic effect to proatherogenic effect.

Metabolic syndrome / Syndrome X is diagnosed if an individual has three or more of the following criteria.[55]

1. Waist circumference >40 inches or >102 cm (men)
   >35 inches or >88 cm(women)
2. Triglycerides > 150 mg/dL
3. HDL cholesterol < 40 mg/dL (men)
   < 50 mg/dL (women)
4. Blood pressure ≥ 130/ ≥85 mm Hg
5. Fasting glucose ≥ 110 mg/dL

Insulin resistance and metabolic syndrome are associated with abdominal obesity. Abdominal obesity measured in terms of waist circumference is a better index for metabolic syndrome than Body mass index.

NON CONVENTIONAL RISK FACTORS:

1. hs CRP

CRP an acute phase reactant is a marker of atherosclerosis process gets elaborated by liver in response to cytokine IL-6. Observational studies found that cells in the atherosclerotic intima also elaborate CRP which itself can enhance prothrombotic response. Values <1 mg/dL is considered as low risk, 1-3 mg/dL intermediate risk, > 3 mg/dL high risk. Individuals with high levels of CRP with low levels of LDL cholesterol are actually found to be at higher risk than those with high LDL and low CRP.[56] This marker is also associated with metabolic syndrome patients in their ischemic episodes.

2. Other markers of inflammation

i) Proinflammatory cytokines- IL-1,IL-6

ii) Soluble forms of cell adhesion molecules - sICAM-1, p- Selectin

iii) Marker of plaque destabilization – myeloperoxidase

iv) Marker of plaque rupture

   a) sCD40 ligand,

   b) Pregnancy Associated Plasma Protein A (PAPP-A)

v) Markers of myocardial dysfunction - ST2 ,a “decoy receptor for IL-33”

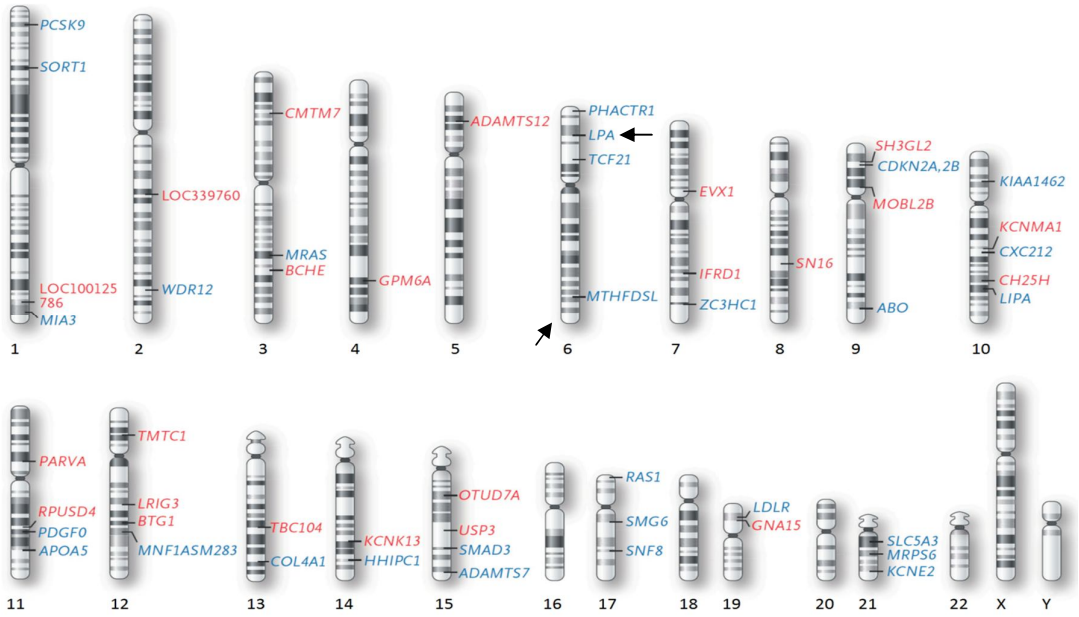
They all have analytical limitations thus limiting its clinical usefulness.

3. Homocysteine

Homocysteine is an intermediate in the conversion of methionine to cysteine. Homocysteine methionine conversion requires the availability of methyl tetrahydofolic acid ,vitamin B_{12} and methionine synthase. Severe homocystinenemia (Homocysteine >100mmol/L) is seen in cystathionine β synthase, vitamin B_6 requiring step. Mild to moderate homocysteinemia is seen in genetic mutation in N^5,N^{10}-methylene tetrahydrofolate reductase (C677T and A1298C Polymorphism). Homocysteine thiolactone is a highly reactive compound that thiolates LDL particles facilitating its aggregation. They are then endocytosed by macrophages thereby potentiating atherogenesis.

4. Genetic markers

Genomic locations of genetic variants associated with myocardial infarction



Courtesy : Braunwald Heart disease chapter 42:Risk markers and primary prevention of cardiovascular disease.

Inheritance plays a significant role in making an individual susceptible to CAD. Genome wide association studies elaborates the singe nucleotide polymorphisms found as a significant risk factor in the general population. [57] The genetic variants responsible for the risk were found to be in the non coding DNA sequences and the patients experiencing early onset of CAD were found to carry 30 known variants. 9p21 risk allele and PCSK9 locus are emerging as a future target for novel therapy. Reynolds risk score includes 7 components for predicting CAD risk.[58,59] This new model included family history of premature CAD and hsCRP in addition to conventional risk factors like age, Total cholesterol, HDL cholesterol, systolic blood pressure and smoking.

5. Environmental exposures:

i) Depression and mental stress:

Negative emotions like depression has a similar effect like that of other major coronary risk factors thereby doubling the risk of developing coronary artery disease. Increased platelet activity, endothelial dysfunction, increased catecholamine levels increasing myocardial oxygen demand, altered cardiac autonomic tone are the mechanisms explaining the contribution of stress to the development of adverse cardiac events.[60,61] In addition to that, adverse life style like consuming poor diet, smoking, physical inactivity, non adherence to medication worsen the condition still more. Work related stress, job strain and effort -reward imbalance are also the sources of vascular risk.

ii) Physical inactivity

Sedentary lifestyle doubles the risk of CHD. Moderate intensity exercise slows down the progression of angiographically proven coronary atherosclerosis in human and lowers the mortality rates independent of other risk factors. Regular exercise can reduce CHD risk by reducing myocardial oxygen demand, by increasing its electrical stability, increasing HDL, reducing obesity and blood pressure and sensitising insulin.

iii) Obesity:

The prevalence of obesity has been in the increasing trend worldwide. Overweight and obese individuals are at risk for developing insulin resistance, hyperinsulinemia, type 2 Diabetes mellitus, Dyslipidemia, Hypertension and Left ventricular hypertrophy.[62] Obesity is found to be an independent risk factor for deaths due to cardiovascular disease in large prospective observational studies.[63] The distribution of body fat influences the development of complications, with centripetal obesity or abdominal obesity having major adverse outcome. Waist hip ratio is a marker for the assessment of centripetal obesity. Studies have found that there is an association between genetic predisposition to obesity and higher consumption of sweetened beverages.[64] Weight reduction improves glucose tolerance, reduces blood pressure and controls dyslipidemia.

iv) Diet

Nutrition transition has taken place according to the changes in the socio economic status of the population. Increased consumption of saturated animal

fats, inexpensive hydrogenated vegetable fats, trans fatty acids, introduction of junk foods, soft drinks and full calorie sugar sweetened beverages contributes to the risk of CHD.

v) Family history of premature CAD:

Premature CAD is defined as incidence of CAD occurring at <55 years of age in males and <65 years of age in females. The chance of developing CAD in future is higher for a person who has a family history of premature CAD in his first degree relatives. When more number of relatives are affected that too occurring at a young age, the next generation developing CAD can be strongly predicted.[65,66] The risk remains unaffected even if other risk factors are nullified.

Causes for premature CAD in young adults: Young CAD- Patients who experience first CAD event < 40 years of age.[67]

1. Atheromatous

- Cigarette smoking
- Positive family H/O premature CAD
- Dyslipidemia- (hypertriglyceridemia and low HDL)
- Impaired Glucose tolerance
- Hyperhomocysteinemia
- Lipoprotein(a)
- Psychosocial stress

Fig 2. Structure of Lipoprotein(a)

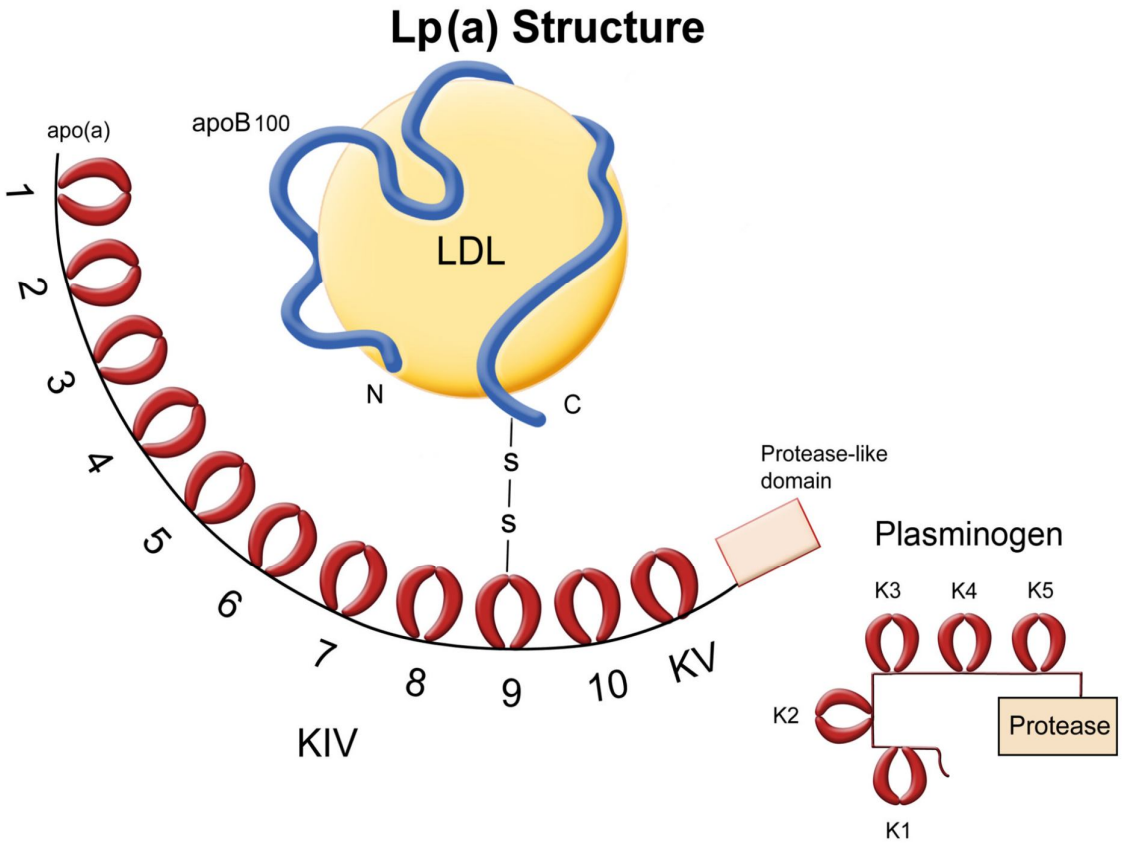


Image courtesy: Metabolism. 2013 April ; 62(4): 479–491.

doi:10.1016/j.metabol.2012.07.024.

2. **Non atheromatous**

- Myocardial bridging (coronary artery tunnelling through myocardium)
- Coronary artery dissection
- Coronary artery aneurysm

3. **Hypercoagulable states**

- Antiphospholipid antibody syndrome
- Nephrotic syndrome
- Factor V leiden mutation
- Contraceptive pill use

4. **Recreational drug abuse**

- Cocaine abuse
- Binge drinking of alcohol

LIPOPROTEIN (a)

The presence of Lipoprotein (a) has been documented in humans and primates including old world and new world monkeys, lesser and greater apes and it is not found to occur in animals that are commonly used in research studies. Liver is the primary site of Lp(a) synthesis, whereas human aorta and carotid artery are the other sites reported to express apo(a) protein.

Structure of Lp(a):

Lipoprotein(a) is a plasma glycoprotein that is structurally similar to LDL, but differs from LDL in having apoprotein(a) linked to apo B100 by means of a disulfide bond.[68] It was first described by Norwegian physician Kaare Berg in the

year 1963.[69] The lipoprotein consists of triglycerides and cholesterol ester enveloped by phospholipids and free cholesterol. The protein part of lipoprotein (a)- apo(a) and apo B100 are present in 1:1 molar ratio.[70]

i) Apo (a) comprises of three domains

- Kringle IV domain
- Kringle V domain
- Inactive serine protease domain

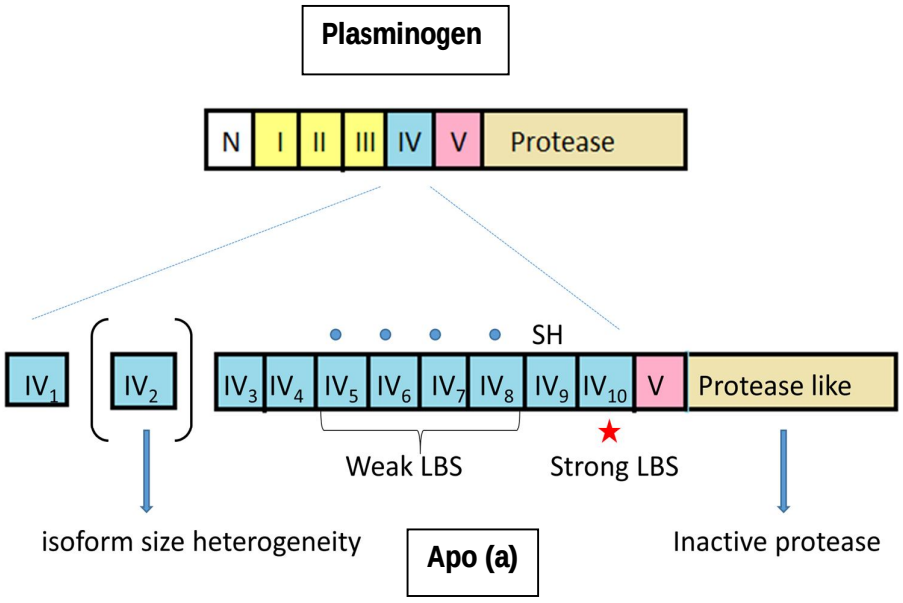
Kringle is a triple loop structural motif which has three internal disulfide bonds. Kringle IV domain comprises 10 different types,[71] in which type 1 and type 3-10 are present in single copy whereas type 2 has variable repeated number of copies (3-40).[72,73] This is due to the variable number of tandem repeats that occurs in apo (a) gene. Because of this variability in kringle IV type 2 domain, apo(a) exihibits high size polymorphism between individuals and even intraindividual size heterogeneity occurs which is the hallmark of lipoprotein(a).

ii) Apo B100 is covalently bound to apo (a) by means of a disulphide bond which involves the unpaired cysteine residue in Kringle IV_9 and unpaired cysteine residue in C terminal of apo B100. Non covalent interactions occur between weak lysine binding sites of kringles IV_5-IV_8 of apo (a) and lysine residues in N terminal of apo B100. [74] This occurs even before the covalent disulfide linkage happens.

Utterman et al [75] identified 6 apo(a) isoforms(F,B,S1-4) using PAGE electrophoresis based on the mobility of isoforms (faster, equal and slower than apo B mobility). Currently there are 34 different apo(a) isoforms based on the differences in their size.[76]

Apo (a) resembles plasminogen structurally

Plasminogen has an N terminal tail which is followed by 5 kringle domains(I-V) and a trypsin like protease domain. Tail region and kringles(I-III) are absent in apo(a).The sequences of kringle IV,V and protease like domain of apo(a) resembles that of plasminogen. Kringle IV has 10 different types each having different aminoacid sequence. Kringle IV_{10} has strong lysine binding site that interacts with fibrinogen.[77]



Transcriptional regulation of *LPA* gene:

The genes coding for Lipoprotein(a) and plasminogen *LPA* and *PLG*, both are present in the same chromosome 6 in humans. They are positioned in head to head configuration separated by intergenic region shared by both genes.

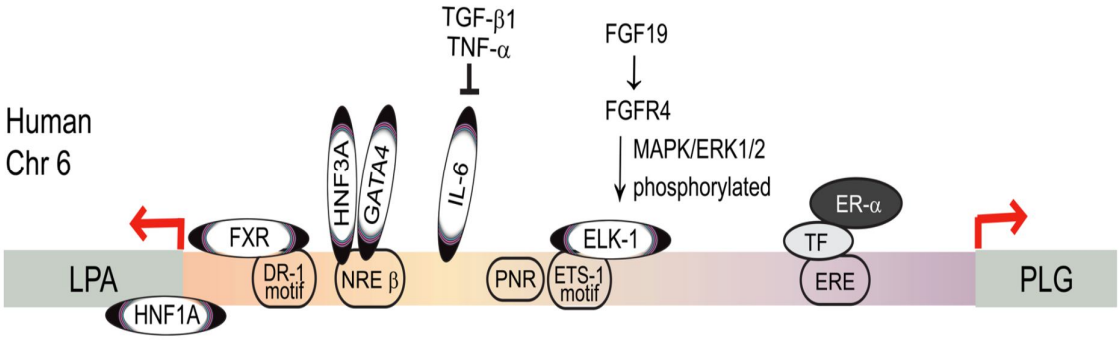
Factors downregulating transcription:

1. Estrogen binds to DNA via negative enhancer estrogen responsive element in apo(a) promoter region and repress apo(a) expression.[78]
2. Transcription factors HNF3A (FOXA1) and GATA4 binds to NRE β negative enhancer region (-1432 to -716 bp) and downregulates apo(a) expression.[79]
3. Farnesoid X receptor (FXR) binds with DR-1 motif located at -826bp region of *LPA*. Bile acids and FXR agonists decreases plasma apo(a) levels.[80]
4. Chennamsetty et al in his study identified Fibroblast growth factor 19 (FGF19) which binds to FGFR4 which promotes the translocation of ELK1 to nucleus where it binds to ETS-1 motif at Lp(a) promoter and inhibiting Lp(a) transcription.

Factors enhancing transcription:

1. HNF1A binds 5' untranslated region of *LPA* gene and transactivates Lp(a) promoter.
2. IL-6 an inflammatory cytokine binds several sites in LPA promoter and acts as a positive regulator.

Transcriptional Regulation of *LPA* gene



Courtesy: Metabolism. 2013 April ; 62(4): 479–491.
doi:10.1016/j.metabol.2012.07.024.

Lp(a) assembly and secretion:

Apo(a) synthesised in ribosomes enters Endoplasmic reticulum where it is folded into kringles with the help of Endoplasmic reticulum chaperones (BiP), calreticulin, calnexin and protein disulfide isomerase. Folded apo(a) either enters golgi apparatus for secretion or directed to proteasome degradation pathway. Assembly of Lp(a) with LDL can occur intracellularly, over the cellular surface or extracellularly. Assembly is complete after establishment of non covalent interaction and covalent linkage between apo(a) and apo B. Assembly inhibitors has been tried to reduce Lp(a) levels but free apo(a) itself contribute to the pathogenesis of atherosclerosis and thrombosis. Therefore agents that affect the apo(a) gene transcription are found to be effective modulators.

Lp(a) catabolism:

Cellular uptake of Lp(a) occurs through endocytotic receptors, however no unique receptor for Lp(a) has been identified. Lp(a) levels is found to be increased

in hypothyroidism and thyroid hormone analogue eprotirome which increases hepatic LDL receptor expression which in turn reduces Lp(a) levels.[81]

Receptor	Location
1. LDL receptor/LRP (LDL receptor related protein)	Liver
2. VLDL receptor	Heart, skeletal muscle and adipose tissue
3. Megalin/gp 330	Epithelial cells, thyroid tissue, proximal tubular cells of kidney, skeletal muscle
4. Plasminogen receptors	Ubiquitous

Apo(a) fragmentation occurs in liver and kidney and the major fragments excreted in urine are N terminal Kringle IV 1,2,3. The enzymes involved in cleavage of apo(a) between kringle IV and Kringle V are

a) neutophil elastase

b) MMP-12 or Macrophage elastase

Lp(a) levels are found to be elevated in kidney disease because apo(a) degradation takes place limitedly. [82]

Vascular uptake of Lp(a):

In humans and other primates Lp(a) accumulates in the atherosclerotic lesions in vessel wall. Lipoprotein (a) binds to various proteins in the extracellular matrix through its apo(a) component or LDL moiety. It is found to

Fig 3. Catabolism of Lipoprotein(a)

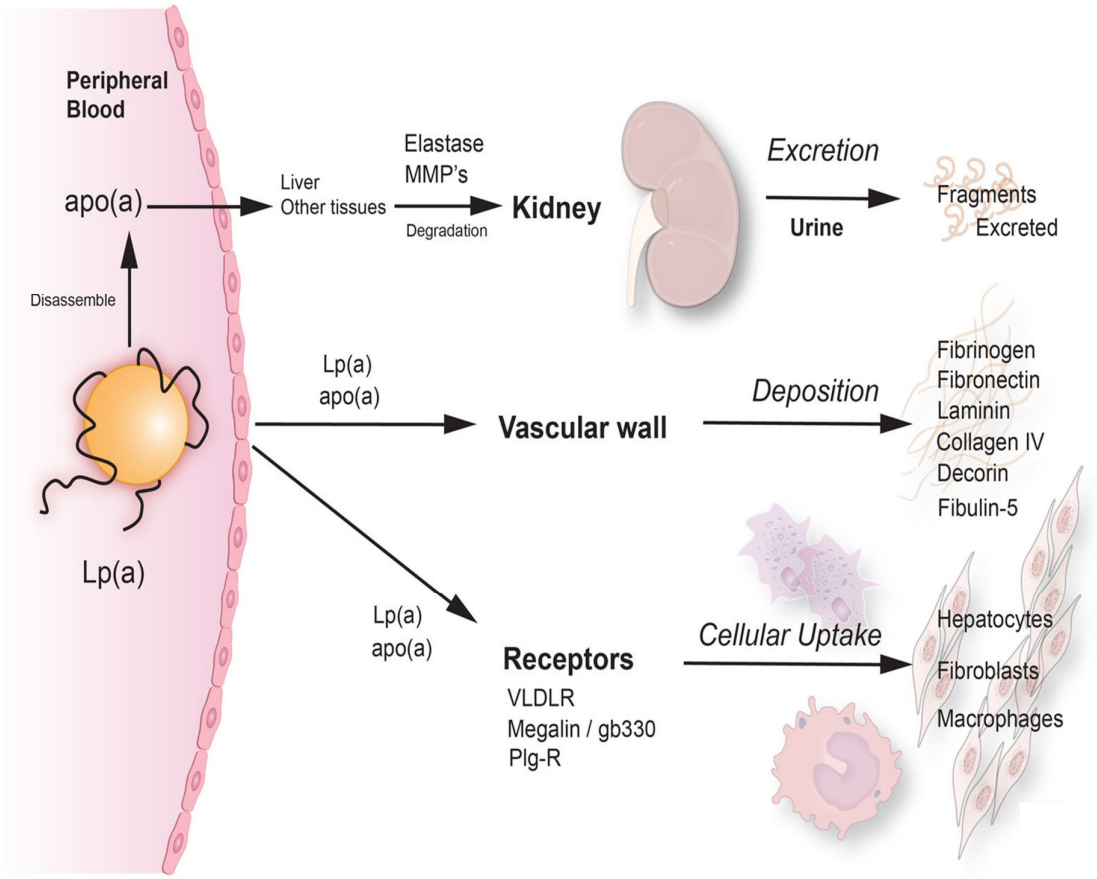


Image courtesy: Metabolism. 2013 April ; 62(4): 479–491.

doi:10.1016/j.metabol.2012.07.024.

bind the proteins with a higher affinity than LDL or plasminogen.[83] The protein in the vessel wall to which it binds are

1. Fibrin- through Kringle V protease domain (competes with plasminogen)
2. Fibronectin- through C terminal region of apo(a)
3. Fibulin-5/DANCE – through N terminal region of apo(a)(kringle IV-type 2 domain).
4. Collagen type-IV
5. Fibrinogen- through kringle IV_{10} (retains Lp(a) in vessel wall)
6. Laminin
7. Defensins

Modifications of Lp(a):

Lp(a) is modified by oxidation and glycation. Oxidised Lp(a) correlates with carotid intimal medial thickness better than Lp(a).[84] Malondialdehyde modified Lp(a) with altered structural and biological properties, is preferentially taken up by the scavenger receptors of macrophages. In hyperglycemia, non enzymatic glycation of Lp(a) occurs but to a lesser extent than LDL.[85] It was also found that apo B100 was more glycated than apoA in Lp(a) particle.

Apo(a) isoform size affects Lp(a) concentration:

Based on the number of kringle 4 type 2 repeats apo(a) isoforms are subdivided into [86]

- Low molecular weight isoform (<22 K-IV repeats)
- High molecular weight isoform (>22 K-IV repeats)

There is inverse correlation between the size of isoform and the plasma lipoprotein (a) levels. This is believed to be due to the difference in the rate of synthesis between the isoforms. Inside the hepatocytes apo(a) is synthesised and processed in Endoplasmic reticulum and the mature form is present in the golgi apparatus. It is then secreted and assembly of apo(a) and apo B 100 takes place at the surface of hepatocytes. Half life of Lipoprotein (a) in circulation is 3-4 days. [87] It is believed that larger isoforms need much retention time in Endoplasmic reticulum for complete processing and this difference in rate of synthesis accounts for lower Lp(a) levels associated with larger isoform and vice versa.[88] Subjects who carry the smaller apo(a) isoform secrete apo(a) at a higher rate and have higher lipoprotein (a) levels.[89]

Mechanisms of Lp(a) pathogenicity: [90]

PROTHROMBOTIC	PROATHEROGENIC
↓ plasmin formation (fibrin) ↓ plasmin formation (pericellular) ↓ TFPI activity ↑ Platelet aggregation Alteration of fibrin clot architecture ↑ EC PAI -1 activity/expression	↑ Smooth muscle cell proliferation or migration ↑ Endothelial cell permeability ↑ Endothelial cell adhesion molecule expression Carrier of Oxidised phospholipids Selective retention in arterial intima ↑ Foam cell formation ↑ Proinflammatory gene expression in EC and macrophages ↑ Lesion calcification ↑ EC monocyte chemotaxis and trans EC migration ↑ EC and macrophage apoptosis

EC- endothelial cell ; PAI-1 plasminogen activator inhibitor-1 ; TFPI- Tissue Factor Pathway Inhibitor

Oxidised lipoprotein(a) is preferentially taken up by the scavenger receptors of macrophages. Lipoprotein(a) inhibits the activation of plasminogen to plasmin which is involved in fibrinolysis.[91] This process of inactivation by apo(a) is by inhibiting the conversion of Glu-plasminogen to Lys-plasminogen which is the appropriate substrate for tissue plasminogen activator. The apo(a) kringle IV_9 domain inhibits the activation of Transforming growth factor β, thereby stimulating the proliferation and migration of smooth muscle cells. Kringle V domain inhibits angiopoietin and vascular endothelial growth factor. In vitro studies demonstrated Lp(a) or apo(a) inhibits tissue factor pathway inhibitor thereby increasing the responsiveness of platelets.[92]

Endothelial dysfunction which is the earliest event in atherosclerosis contributed by apo (a) kringle IV_{10} domain through its strong lysine binding site stimulates

1. Rho/ Rho Kinase/ MYPT1 dependant signalling pathway (cytoskeletal rearrangement resulting in endothelium contraction & endothelial permeability occurs)[93]
2. The expression of endothelial cell adhesion molecule enhancing the endothelial permeability which when associated with high LDL cholesterol potentiates its retention in vascular wall. [94]

Recently it has been proposed that apo(a) component of Lp(a) is involved in **β catenin** pathway where it dissociates from its cell surface complexes, enters the nucleus and increases the expression of gene coding COX-1 (cycloxygenase-1), an inflammatory mediator. Synthesis and secretion of prostaglandin E is

enhanced. Apo(a) is thus considered as the modulator of phenotype of vascular endothelial cells.[95]

Oxidised phospholipids (OxPLs) are proinflammatory particles that are present in atherosclerotic lesions which mediates the phenotype conversion of macrophages to foam cells.[96] It has been demonstrated that association of OxPLs with Lp(a) is more than its association with free LDL.[97] Edensteil et al demonstrated Lp(a) is covalently bound to OxPLs through lysine residues in apo(a) kringle V domain in a Schiff base linkage . This lysine phosphotidyl choline adducts impart proinflammatory character to apo(a). Kringle V domain is also found to mediate the release of IL-8 from cultured human macrophages.[98] In the Dallas heart study, OxPL/ apo B levels correlated strongly with the Lp(a) levels and it was inversely correlating with apo(a) isoform size.[99] Eleveated OxPL/ apo B level is related to progressive coronary and carotid atherosclerosis and poorer cardiovascular outcome. Lp(a) is a carrier of OxPLs. Lp(a) when present in low physiological concentrations in plasma binds and sequesters proinflammatory OxPL along with it but when it is present in high pathological concentration, it delivers OxPL to the site of atherosclerosis.[100] Lp(a) induces CD36-TLR2 mediated apoptosis of macrophages and endothelial cells under Endoplasmic reticulum stress.

Secreted **Phospholipase A_2** ($sPLA_2$) and lipoprotein associated PLA_2 (Lp-PLA_2) are the markers of CAD risk. The enzyme cleaves phospholipids into lysophospholipid and free fatty acids. PLA_2 interacts with OxPL/ apo B and it is

found that CAD risk is potentiated when there is increased OxPL/ apo B level along with increased concentration of both forms of $PLA_{2.}$ [101]

Lp(a) is found in the advanced human atherosclerotic lesions with asscociated areas of **calcification**. When smooth muscle cells are cultured, Lp(a) is found to calcify it by stimulating the uptake of calcium by these cells and promoting the change in phenotype showing osteoblastic activity. It is evidenced by upregulated osteoblast specific factor-2 and alkaline phosphatase expression. The presence of Lp(a) within arterial wall provoking any early change in the vessel wall before any visible manifestations of atherosclerosis is noted is being questioned. The proteomic analysis of proteins in aortic wall reveals protein expression reflecting the effect of Lp(a) on cytoskeleton, redox state, cell adhesion etc.[102] Moreover Lp(a) affects the catabolism of other lipoproteins. It has been noted that apo a kringle IV_{5-8} inhibits the clearance of non HDL cholesterol. Therefore Lp(a) adds up the burden in previously known hypercholesterolemic patients.[103]

Independent role of apo(a) size in assessing risk:

Whether apo(a) size determination predicts cardiovascular risk independent of lipoprotein(a) concentration and the pathogenic mechanism for its direct contribution CAD is still unclear. However it is found that

1. Smaller apo(a) isoform preferentially binds vascular intima
2. Smaller isoforms binds strongly to fibrin and inhibits fibrinolysis [104](inhibition of activation of plasminogen to plasmin)

Genetic factors affecting Lp(a) concentration:

As mentioned above previously, the size of *LPA* gene that contributes to the size of the isoforms reflects 60% of variation in plasma lipoprotein(a) levels.[105] However this relationship with respect to apo(a) isoform size cannot explain the Lp(a) concentration in an individual completely. This is because African Americans have high Lp(a) levels inspite of having larger isoforms.[106] Genetic variants either in *LPA or PLG* gene contribute to 20-30% of variations in Lp(a) concentration. Most common variants found are

i) ($TTTTA_n$) Pentanucleotide repeat polymorphism in the 5' flanking region of *LPA* gene

ii) Single Nucleotide Polymorphisms rs10455872 (intronic region) and rs3798220 (missense mutation causing Ile → met substitution at position 4399)

Recently, 10 SNPs have been identified contributing to 70% difference in Lp(a) between Africans and Europeans.[107]

Factors influencing Lp(a) levels:

I) Transcription : ($TTTTA_n$) Pentanucleotide repeat polymorphism

rs10455872 } SNP
rs3798220 }

II) Posttranslation modification

(intracellular processing and folding in ER)

- Size of LPA allele (isoform size)

III) **Lp(a) assembly:** smaller isoforms can covalently bind apo B100 more efficiently.

Lipoprotein(a) and premature CAD:

Lipoprotein (a) is an independent hereditary risk factor for the development of myocardial infarction occurring in men < 45 years of age.[108] Various studies report that Asian Indians have the highest Lp(a) levels irrespective of their migration status to other countries,[109-113] supporting the evidence of genetic predisposition. Lipoprotein (a) increases the risk of atherogenicity 10 times as that of LDL.[114] The risk is magnified when it is associated with high LDL cholesterol and TC/HDL ratio. The levels of lipoprotein(a) is found to correlate with the severity of the score in coronary angiogram.[115] Lp(a) levels attain the adult concentration in plasma by 8 months of age and therefore the pathogenesis of atherosclerosis starts 15-20 years earlier than other risk factors start contributing to the CAD thereby precipitating premature CAD. The levels of Lp(a) in plasma remains stable and it is not altered by age, gender and environmental factors. [116]

According to European Atherosclerosis Society individuals who should be considered for lipoprotein(a) screening[117]

Individuals with:

- Premature CAD.
- Familial hypercholesterolaemia.
- A family history of premature CAD and/or elevated Lp(a).

- Who respond poorly to statin in LDL-C lowering
- Recurrent CAD despite patient being on optimal hypolipidemic drugs.
- ≥5% 10-year risk of fatal CAD according to SCORE (Systematic Coronary Risk Evaluation)

Challenges in Lp(a) measurement:

Lp(a) is not currently measured along with routine lipid profile because methods of measurement is technically challenging. To increase the predictive power of Lp(a), standardised methods of measurement should be available to accurately measure its levels in plasma. The hindrances in measurement are

1. Apo (a) size heterogeneity due to variable number of copies of Kringle IV_2
2. Apo (a) association with apo B100
3. Sequence homology between apo(a) and plasminogen

When antibodies to apo (a) Kringle IV_2 is used in immunoassays interferences from size heterogeneity occurs. Samples whose apo(a) size matches that of apo (a) size in calibrator does not affect the Lp(a) levels. Larger and smaller isoform size compared to the calibrator used results in overestimation and underestimation of Lp(a) levels respectively. This can lead to misclassification of patients under risk estimation.

Guidelines for measurement of Lipoprotein(a)[118]

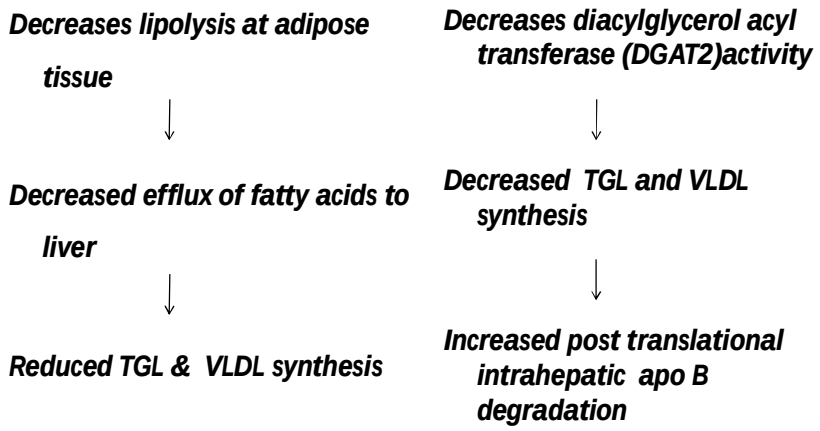
1. Antibodies directed to epitope other than kringle IV_2 to be used. ELISA method that uses antibodies to kringle IV_9 is recognised as reference method for Lp(a) assay.
2. Lp(a) levels should not be measured in terms of mass i.e. in mg/dL, mg/L (which also reflects the lipid and carbohydrate content) instead should be expressed in nmol/L so that it allows data comparable directly from different studies.
3. The WHO approved IFCC secondary reference material with assigned value of 107 nmol/L should be used for calibrating assays.
4. If the laboratory use method that is sensitive to isoform size, then samples whose values greater than 50 nmol/L should be sent to referral laboratories which uses validated methods.
5. Because there is an impact in the measurement of Lp(a), conditions for collection and storage of samples should be determined for individual assays.

Therapeutic management of high Lp(a)

Unlike other lipoproteins, there is no approved pharmaceutical agent that specifically brings down Lp(a) level without affecting other lipoproteins. Non pharmacological measures like diet and exercise have been found to have no effect on modulating Lp(a) levels in plasma. This is due to the strong genetic determination of *LPA* gene encoding apo(a). To date, plasma apheresis, high dose niacin therapy (sustained/extended release,1-2 g/day) and aspirin has been

approved for reducing Lp(a) concentration. Niacin(nicotinic acid) works in two ways:

Mechanism of action of Niacin:



Adverse effects of niacin therapy includes flushing, upper GI distress, hyperglycemia, hyperuricemia, hepatotoxicity. Administration is absolutely contraindicated in Chronic liver disease and in patients with severe gout.

Therapeutic management of elevated Lp(a)[119]

Agent	Mechanism of action	Therapeutic status
Reduces apo (a)		
1.Estrogen	Acts on HRE in *LPA* promoter. Lp(a) decreases with HRT	NR
2.Anabolic steroids	Acts on gene expression	NR
3.Toclizumab	IL-6 receptor antagonist	approved
4.FGF-19	*LPA* gene repression	Preclinical
5.FXR	Bile acid activated receptor, transactivation of FGF-19	Preclinical
6.Aspirin	Reduces *LPA* expression	Approved
Reduces apo B synthesis		
7.Mipomersen	Antisense nucleotide decreasing apoB synthesis	Phase III
8.Apo B peptides	Inhibit Lp(a) assembly	Preclinical
Reduces Lipid		
9.Apheresis	LDL and Lp(a) removed from circulation	Approved
10.Statins	HMG CoA reductase inhibitor reduces cholesterol synthesis and increases hepatic LDL receptors	Approved
11.Niacin	As mentioned above	Approved
12.Anacetrapib	Cholesterol ester transfer protein inhibitor raises HDL and lowers LDL cholesterol	Phase III
13.Eprotirome	Thyromimetic. Increased expression of hepatic LDL receptor	Clinical trial discontinued
14.PCSK9 Inhibitor	PCSK9 promotes intracellular LDL receptor degradation. Inhibitor decreases Lp(a).	Phase II
15. PUFA , carnitine	Long term consumption	Diet supplement

NR- not recommended

Aims & Objectives

AIM OF THE STUDY

1. To estimate the level of plasma Lipoprotein(a) in angiographically proven young coronary artery disease patients and in age and sex matched healthy controls.

2. To correlate Lipoprotein(a) concentration of Coronary Artery Disease patients with their first degree relatives.

3. To correlate the concentration of Lipoprotein (a) with the severity of Coronary Heart Disease.

Materials & Methods

MATERIALS AND METHODS

STUDY DESIGN:

This is a case control study and the study protocol was approved by the Institutional Ethics Committee of Madras Medical College, Chennai. Study has been conducted at Institute of Biochemistry & Institute of Cardiology, Rajiv Gandhi Government General Hospital, Chennai which is attached to Madras Medical College.

STUDY PERIOD : October 2016- April 2017

INCLUSION CRITERIA:

Group A - Angiographically proven young coronary artery disease patients (both males and females) aged <45 years

Group B - First degree relatives of group A (siblings, children)

Group C - Age and sex matched healthy controls

EXCLUSION CRITERIA:

- Recent Myocardial infarction (<6 weeks)
- Patients with Liver diseases.
- Chronic kidney disease
- Patient with acute illness; infection.
- Familial hypercholesterolemia
- Thyroid disorders
- Patients on high dose niacin therapy

SAMPLE COLLECTION:

With the informed consent from the subjects, 4ml of blood K_2-EDTA sample was collected after 12 hours of fasting. The sample was centrifuged and plasma was aliquoted. The following investigations were done.

Tube	Anticoagulant	Volume of blood	Investigations
Tube 1	K_2-EDTA	2 mL	Fasting Plasma Glucose, Fasting Lipid Profile
Tube 2	K_2-EDTA	2 mL	Lipoprotein(a)

The aliquoted plasma was stored immediately at - 80^{o}C

ESTIMATION OF FASTING PLASMA GLUCOSE

Method:

Hexokinase method

Principle:

Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ Glucose-6-phosphate + ADP

Glucose-6-phosphate + NADP + $\xrightarrow{\text{G6PD}}$ Gluconate-6-phosphate + NADPH + H^+

G6PD -Glucose-6-phosphate dehydrogenase

NADPH formed is measured at 340nm.

Reagent Composition:

ACTIVE INGREDIENTS	CONCENTRATION
R1: MES buffer	5 mmol/L
Mg^{2+}	24 mmol/L
ATP	>4.5 mmol/L
NADP	>7.0 mmol/L
R2: HEPES buffer	200 mmol/L
Mg 2+	4 mmol/L
Hexokinase (Yeast)	>300 μkat/L
Glucose-6-phosphate dehydrogenase(E.coli)	>300 μkat/L

Calibrator

Glucose 100 mg/dL

Assay Parameters:

MODE	End point
Wavelength 1 (nm)	340
Wavelength 2 (nm)	700
Sample volume (µL)	2 µL
Reagent volume (µL)	R1- 28 µL , R2-10 µL
Incubation time (min)	10
Incubation temperature (^{o}C)	37
Normal low (mg/dL)	74
Normal high (mg/dL)	109
Linearity low (mg/dL)	2.0
Linearity high (mg/dL)	750.0
Blank with	Reagent
Absorbance limit(max)	0.2
Units	mg/dL

Reference Interval:

Fasting plasma glucose – 70-110 mg/dL

LIPID PROFILE:

ESTIMATION OF PLASMA TOTAL CHOLESTEROL

Method:

CHOD-PAP METHOD (Cholesterol oxidase-phenol-aminophenazone peroxidase)

Principle:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2\,H_2O_2 + 4\text{AAP} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red quinine-imine dye} + 4\,H_2O$$

Reagent Composition:

ACTIVE INGREDIENTS	CONCENTRATION
PIPES Buffer (pH– 6.8)	225 mmol/L
Mg^{2+}	10 mmol/L
Sodium cholate	0.6 mmol/L
Phenol	≥12.6 mmol/L
4-aminophenazone	≥ 0.45 mmol/L
Fatty alcohol polyglycol ether	3%
Cholesterol esterase (Pseudomonas)	≥25 µkat/L (≥1.5 U/m L)
Cholesterol oxidase (E.coli)	≥ 7.5µkat/L (≥0.45 U/m L)
Peroxidase (horseradish)	≥12.5µkat/L (≥0.75 U/m L)

Calibrator

Total Cholesterol 164.5 mg/dL

Assay Parameters:

MODE	End point
Wavelength 1 (nm)	505
Wavelength 2 (nm)	700
Sample volume (µL)	2
Reagent volume (µL)	47 (+ 93µ L Diluent H_2O)
Reaction time/ Assay points	10/57
Normal low (mg/dL)	<200
Normal high (mg/dL)	>200
Linearity low (mg/dL)	3.86
Linearity high (mg/dL)	800
Blank with	Reagent
Absorbance limit(max)	0.2
Units	mg/dL

Reference Interval: [120]

Total cholesterol

Desirable <200 mg/dL

Borderline high 200-239 mg/dL

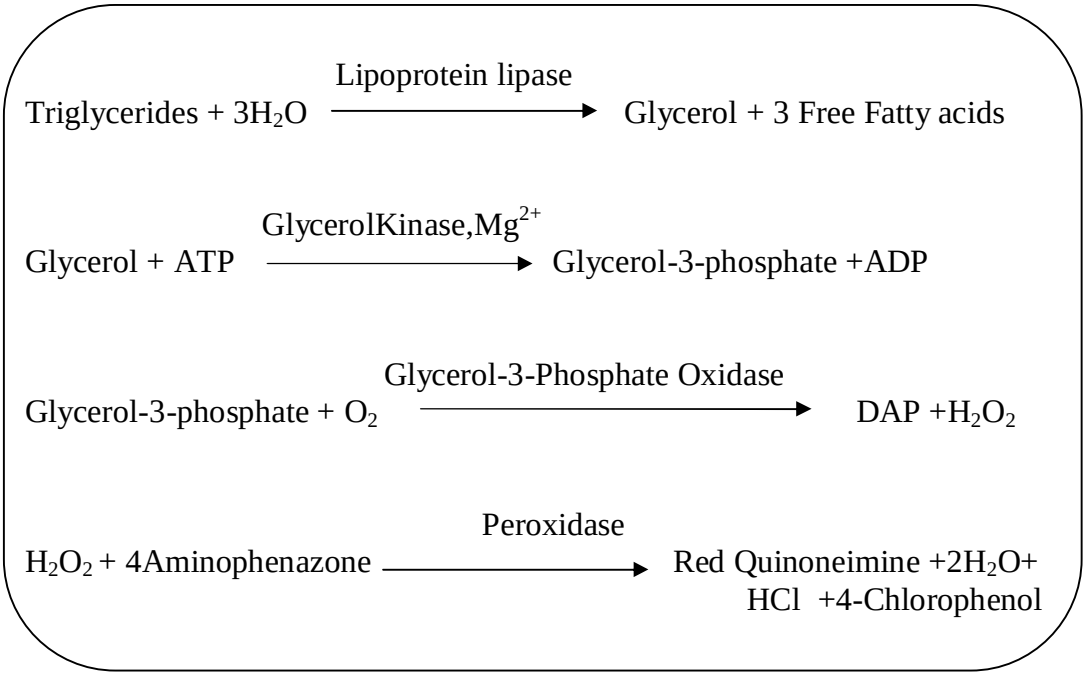
High ≥ 240 mg/dL

ESTIMATION OF PLASMA TRIGLYCERIDES

Method:

GPO-PAP method (glycerophosphate oxidase- phenol- aminophenazone peroxidase)

Principle:



Reagent Composition:

ACTIVE INGREDIENT	CONCENTRATION
PIPES buffer (pH 6.8)	50 mmol/L
Sodium cholate	0.20 mmol/L
ATP	≥1.4 mmol/L
Mg^{2+}	40mmol/L
4-aminophenazone	≥0.13 mmol/L
4-chlorophenol	4 .7mmol/L

Lipoprotein lipase (Pseudomonas)	≥83 µkat/L
Glycerokinase(Bacillus stearothermophilus)	≥3 µkat/L
Glycerol phosphate oxidase (E.coli)	≥41 µkat/L
Peroxidase (horse radish)	≥1.6 µkat/L

Calibrator

Triglycerides -137.6 mg/dL

Assay Parameters:

Mode	END POINT
Wavelength 1 (nm)	505
Wavelength 2 (nm)	700
Sample volume (µL)	2
Reagent volume (µL)	120 (+ 28 µL Diluent H_2O)
Reaction time/Assay points	10/57
Normal low (mg/dL)	0
Normal high (mg/dL)	150
Linearity low (mg/dL)	8.85
Linearity high (mg/dL)	885
Blanking with	Reagent
Absorbance limit (max)	0.5
Units	mg/dL

Reference interval:

Triglycerides: [120]

Normal	<150 mg/dL
Borderline High	150-199 mg/dL
High	200-499 mg/dL
Very high	≥500 mg/dL

ESTIMATION OF PLASMA HDL CHOLESTEROL

Method:

PEG- Cholesterol esterase - oxidase method (Direct method)

Principle:

PEG- cholesterol esterase

HDL- Cholesterol esters + H_2O ⟶ HDL -cholesterol+ RCOOH

PEG- cholesterol oxidase

HDL –cholesterol + O_2 ⟶ Δ^4 -cholestenone + H_2O_2

Peroxidase

2 H_2O_2 + 4- amino-antipyrine ⟶ purple blue pigment + $5H_2O$

+HSDA* + H^+ + H_2O

*HSDA- Sodium N-(2- hydroxyl -3- sulfopropyl)-3,5-dimethoxyaniline

Chylomicrons, VLDL & LDL are resistant to PEG modified enzymes.

Calibrator:

HDL –C 25 mg/dL

Reagent composition:

ACTIVE INGREDIENTS	CONCENTRATION
R1 HEPES Buffer	10.07 mmol/L
CHES (pH-7.4)	96.95 mmol/L
Dextran sulfate	1.5g/L
Magnesium nitrate hexahydrate	>11.7 mmol/L
HSDA	0.96 mmol/L
Ascorbate oxidase	>50 µkat/L
Peroxidase (horseradish)	>16.7 µkat/L
R2 HEPES Buffer (pH-7.0)	10.07 mmol/L
PEG -Cholesterol esterase (Pseudomonas)	>3.33 µkat/L
PEG-Cholesterol oxidase (Streptomyces)	>127µkat/L
Peroxidase (horseradish)	>333µkat/L
4-amino-antipyrine	2.46 mmol/L

Assay Parameters:

Mode	2 -point End
Wavelength 1 (nm)	600
Wavelength 2 (nm)	700
Sample volume (µL)	2.5
Reagent Volume (µL)	R1- 150 R2- 50

Reaction time/ Assay points	10/ 6-33
Normal low (mg/dL)	<40
Normal high (mg/dL)	≥60
Linearity low (mg/dL)	3
Linearity high (mg/dL)	121
Blank with	Reagent
Absorbance limit (max)	0.2
Unit	mg/dL

Reference interval:

HDL-C [120]

Low <40 mg/dL

High ≥60 mg/dL

VLDL & LDL CHOLESTEROL

They are calculated using Friedewald's formula. It is given by:

LDL – C = TC-(HDL-C+VLDL-C)

VLDL-C = TGL/5

The above formula holds for triglycerides value less than 400 mg/dL .

Reference Interval:

LDL cholesterol [120]

Optimal	< 100 mg/dL
Near optimal	100 to 129 mg/dL
Borderline high	130 to 159 mg/dL
High	160 to 189 mg/dL
Very high	≥ 190 mg/dL

ESTIMATION OF PLASMA LIPOPROTEIN (a):

Method:

Immunoturbidimetry

Principle:

Lp(a) in the sample agglutinates with anti Lp(a) antibody which is adsorbed to latex particles .The change in absorbance is measured at 700 nm.

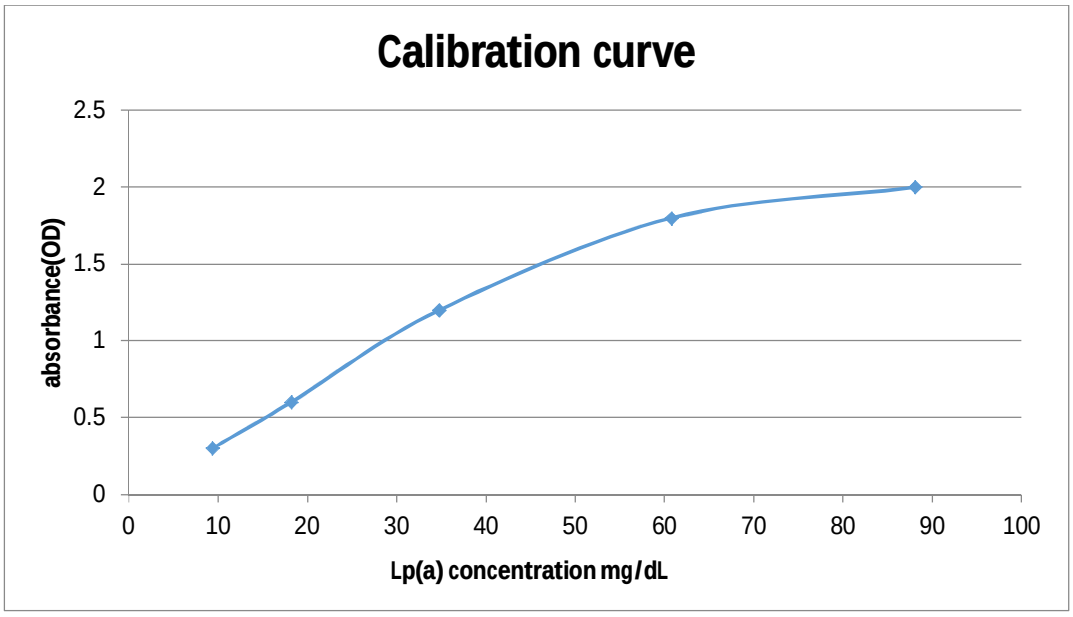
Reagent Composition:

Contents	Concentration
R1 Buffer	
Glycine	0.17M
Sodium chloride	1.08M
Sodium ethylenediamine Tetra acetic acid disodium salt dihydrate	0.05M
Sodium azide	<0.09% w/v
R2 Latex reagent	
Glycine	0.17M
Sodium chloride	0.1M
Latex particles suspension coated with anti Lp(a) antibodies	0.5%
Sodium azide	<0.09% w/v

Calibrator

5 level calibrator is used. The values assigned to each level are traceable to recommended WHO Reference material SRM2B.

	Lipoprotein(a) mg/dL	OD(absorbance)
Level 1	9.4	0.3
Level 2	18.2	0.6
Level 3	34.8	1.2
Level 4	60.8	1.8
Level 5	88.1	2.0



Assay Parameters:

MODE	Fixed
Wavelength	700nm
Sample volume (µL)	3
Diluent volume	10
Reagent volume R1 (µL)	100
Reagent volume R2 (µL)	50
Reaction type	Increase
Point 1	13
Point 2	27
Incubation temperature (^{o}C)	37
Normal low (mg/dL)	0
Normal high (mg/dL)	30
Linearity low (mg/dL)	2
Linearity high (mg/dL)	90
Minimum OD	-0.1
Maximum OD	2.5
Reagent OD limit	-0.1 (low)
	2.5 (high)
Units	mg/dL
Calibration Type	6AB
Formula	(0)SPLINE
Count	2
Prozone effect	>341mg/dL

Reference Interval:

Adults < 30 mg/dL.

This cut off point is based on the study conducted in Caucasian reference population.[121] European atherosclerosis society recommends the desirable level as <50 mg/dL.[122]

NHLBI (National Heart Lung and Blood institute) recommends to express Lp(a) values in number of particles (nmol/L) rather than expressing in terms of mass. According to Framingham data, values more than 75 nmol/L is considered as high risk.[123]

Conversion factor:[124] $\text{nmol/L} = \frac{\text{value in mg/dL}}{0.4167}$

However the reference range for Lp(a) values will change according to race and ethnicity, it is advisable for the laboratories to establish its own reference range according to age, sex, location of the study population.

Statistical Analysis

STATISTICAL ANALYSIS

1. Statistical analysis was done using SPSS software (IBM SPSS Statistics 20)

2. There were three groups analysed

   Group A (30) - Angiographically proven young coronary artery disease patients (both males and females) aged <45 years

   Group B (30) - First degree relatives of group A (siblings, children)

   Group C(30) - Age and sex matched healthy controls

3. Chi square test (2*3 table) was used to analyse categorical variables like sex(male/female), diabetes mellitus (yes/no), hypertension (yes/no), smoking (yes/no), alcohol(yes/no) and family history of premature CAD(yes/no) between three groups.

4. ANOVA (Analysis of variance) was used to compare continuous variables like waist hip ratio, Total cholesterol, HDL cholesterol, TC:HDL cholesterol ratio, Triglycerides, LDL and Lp(a) values.Microsoft excel spreadsheet was used to represent graphically the distribution of family H/O premature CAD and Lp(a) concentration between cases, first degree relatives and controls.

5. Using SPSS software, true positive(sensitivity) and false positive (1- specificity) rates at specific Lp(a) cut offs and Receiver Operating Characteristics Curve were obtained. Area under the curve, 95% confidence interval, standard error were also obatained.

6. Pearson correlation analysis was done to establish correlation between

   a) Lp(a) levels in cases with their first degree relatives

   b) Lp(a) levels in cases with their coronary angiogram- CAG findings.

   c) Lp(a) with Fasting plasma Glucose, Total cholesterol, HDL-C, Triglycerides, LDL in all the three groups.

7. Microsoft excel 2007 was used to graphically illustrate the distribution of Lp(a) levels between subjects, correlation between Lp(a) concentration between cases and first degree relatives, Lp(a) levels in cases with their coronary angiogram- CAG findings.

8. Stepwise regression analysis was carried out to show the independent predictors of CAD risk.

Results

MASTER CHART

ID / S. No	AGE	SEX	DM	HTN	SMOKE	ALCOHOL	f H/o PCAD	WAIST HIP RATIO	CAG FINDING	CAG IMPRESSION	FPG	TC	HDL	TC:HDL RATIO	TGL	LDL	Lp(a)
CASES											mg/dL	mg/dL	mg/dL		mg/dL	mg/dL	mg/dL
1	36	F	YES	NO	NO	NO	YES	0.8	80%	DVD	74	153	31	4.94	100	102	80.5
2	42	M	YES	YES	YES	NO	NO	0.97	50%	DVD	113	147	42	3.50	90	87	61.1
3	41	M	YES	YES	NO	NO	NO	0.98	30%	SVD	175	127	30	4.23	77	81.6	7.1
4	45	M	YES	YES	YES	NO	YES	0.96	70%	SVD	83	154	39	3.95	211	72.8	51.8
5	40	M	NO	YES	YES	NO	NO	1	80%	TVD	101	196	28	7.00	101	147.8	89.1
6	30	M	NO	NO	NO	NO	YES	0.98	95%	DVD	76	192	30	6.40	64	149.2	81.1
7	42	M	NO	NO	YES	NO	NO	0.98	50%	SVD	73	148	34	4.35	144	85.2	26.2
8	40	M	NO	NO	NO	NO	NO	1.1	30%	SVD	84	174	34	5.12	103	119.4	10.4
9	40	M	NO	YES	NO	NO	NO	1.03	50%	SVD	120	127	35	3.63	379	16.2	24.9
10	42	M	NO	NO	NO	NO	YES	0.9	70%	SVD	202	217	35	6.20	216	138.8	7.6
11	33	M	YES	NO	NO	NO	YES	0.95	80%	DVD	162	169	36	4.69	100	113	58.9
12	43	M	NO	NO	YES	YES	NO	0.9	50%	SVD	100	218	24	9.08	172	159.6	5.9
13	45	M	NO	NO	YES	YES	NO	0.95	50%	SVD	101	106	30	3.53	47	66.6	45.2
14	40	M	NO	NO	NO	YES	YES	0.9	95%	DVD	98	212	35	6.06	60	165	61.9
15	25	M	YES	NO	NO	NO	NO	0.9	50%	SVD	351	177	26	6.81	97	131.6	29.7
16	34	M	YES	NO	YES	NO	YES	0.98	70%	DVD	190	183	28	6.54	175	120	66.7
17	35	M	NO	NO	NO	YES	NO	0.96	70%	DVD	74	220	35	6.29	53	174.4	62.3
18	39	M	YES	YES	YES	YES	YES	0.92	50%	SVD	113	153	21	7.29	142	103.6	40.1
19	41	M	NO	YES	NO	NO	YES	0.95	30%	SVD	76	154	24	6.42	89	112.2	19
20	48	F	YES	YES	NO	NO	NO	0.94	30%	SVD	87	137	22	6.23	106	93.8	11
21	37	M	NO	NO	NO	NO	NO	0.94	30%	SVD	88	221	32	6.91	168	155.4	10.8
22	37	F	YES	NO	NO	NO	NO	0.88	30%	SVD	161	218	31	7.03	273	132.4	17.7
23	41	M	NO	NO	NO	NO	NO	0.97	50%	SVD	86	154	37	4.16	129	91.2	10.1
24	34	F	YES	NO	NO	NO	YES	0.95	30%	SVD	328	160	25	6.40	149	105.2	18
25	38	M	YES	NO	YES	YES	YES	0.94	30%	SVD	215	178	32	5.56	157	114.6	8.2
26	43	M	NO	YES	NO	YES	YES	0.98	95%	DVD	101	198	36	5.50	68	148.4	71.9
27	37	M	NO	NO	YES	YES	NO	0.95	95%	TVD	97	132	39	3.38	134	66.2	88
28	37	M	NO	NO	YES	YES	NO	0.96	30%	SVD	93	230	29	7.93	47	191.6	15.5
29	37	M	NO	NO	NO	NO	NO	1.05	30%	DVD	93	169	29	5.83	47	130.6	18.4
30	41	M	NO	NO	NO	YES	NO	0.94	30%	SVD	99	106	32	3.31	213	31.4	11

ID / S. No	AGE	SEX	DM	HTN	SMOKE	ALCOHOL	f H/o PCAD	WAIST HIP RATIO	CAG FINDING	CAG IMPRESSION	FPG	TC	HDL	TC:HDL RATIO	TGL	LDL	Lp(a)
CASES											mg/dL	mg/dL	mg/dL		mg/dL	mg/dL	mg/dL
FIRST DEGREE RELATIVES																	
1	45	F	YES	NO	NO	NO	NO	0.82	NA	NA	174	223	34	6.56	219	145.2	28.9
2	15	M	NO	NO	NO	NO	NO	0.92	NA	NA	86	154	30	5.13	106	102.8	53.9
3	58	M	NO	NO	NO	NO	NO	0.93	NA	NA	88	212	37	5.73	115	152	9.9
4	18	M	NO	NO	NO	NO	NO	0.92	NA	NA	99	169	33	5.12	144	107.2	25.2
5	51	M	YES	NO	NO	NO	NO	0.96	NA	NA	206	240	47	5.11	138	165.4	14.5
6	28	M	NO	NO	NO	NO	YES	0.92	NA	NA	92	157	31	5.06	161	93.8	9.7
7	15	F	NO	NO	NO	NO	NO	0.9	NA	NA	86	154	40	3.85	106	92.8	52.9
8	12	M	NO	NO	NO	NO	NO	0.9	NA	NA	90	140	28	5.00	121	87.8	5.6
9	18	M	NO	NO	NO	NO	NO	0.9	NA	NA	82	208	36	5.78	202	131.6	20.1
10	34	M	NO	NO	NO	NO	YES	0.9	NA	NA	89	178	23	7.74	139	127.2	14
11	19	M	NO	NO	NO	NO	NO	0.92	NA	NA	68	141	38	3.71	100	83	32.1
12	17	M	NO	NO	NO	NO	NO	1	NA	NA	91	115	37	3.11	91	59.8	8
13	25	M	NO	NO	YES	NO	NO	0.92	NA	NA	88	269	44	6.11	138	197.4	32.5
14	20	F	NO	NO	NO	NO	NO	1	NA	NA	91	185	40	4.63	98	125.4	35
15	40	M	YES	NO	NO	NO	NO	0.9	NA	NA	212	206	30	6.87	150	146	19.6
16	26	M	NO	NO	YES	NO	NO	0.98	NA	NA	81	155	36	4.31	101	98.8	31.6
17	50	F	NO	NO	NO	NO	NO	0.88	NA	NA	90	189	35	5.40	105	133	76.4
18	36	M	NO	NO	NO	NO	NO	0.98	NA	NA	69	139	44	3.16	102	74.6	51.4
19	39	M	NO	NO	NO	NO	NO	0.97	NA	NA	88	130	34	3.82	130	70	11.5
20	44	F	NO	NO	NO	NO	NO	0.95	NA	NA	80	130	35	3.71	130	69	11.5
21	14	M	NO	NO	NO	NO	NO	0.88	NA	NA	84	180	35	5.14	115	122	8.1
22	48	F	YES	NO	NO	NO	NO	0.89	NA	NA	283	283	43	6.58	250	190	29.6
23	47	M	NO	NO	NO	NO	NO	0.98	NA	NA	90	185	37	5.00	120	124	54.7
24	16	M	NO	NO	NO	NO	YES	0.97	NA	NA	84	168	42	4.00	90	108	29.1
25	18	M	NO	NO	NO	NO	YES	0.95	NA	NA	88	110	36	3.06	129	48.2	7.6
26	20	M	NO	NO	NO	NO	YES	0.95	NA	NA	90	132	39	3.38	76	77.8	37.2
27	53	M	NO	NO	YES	YES	NO	0.9	NA	NA	81	181	46	3.93	101	114.8	46.7
28	18	M	NO	NO	NO	NO	NO	0.9	NA	NA	90	121	35	3.46	88	68.4	20
29	35	M	NO	NO	NO	NO	NO	0.95	NA	NA	90	121	35	3.46	98	66.4	20.4
30	14	F	NO	NO	NO	NO	NO	0.88	NA	NA	78	201	40	5.03	94	142.2	11.8

ID / S. No	AGE	SEX	DM	HTN	SMOKE	ALCOHOL	f H/o PCAD	WAIST HIP RATIO	CAG FINDING	CAG IMPRESSION	FPG	TC	HDL	TC:HDL RATIO	TGL	LDL	Lp(a)
CASES											mg/dL	mg/dL	mg/dL		mg/dL	mg/dL	mg/dL
CONTROLS																	
1	32	M	NO	NO	NO	NO	NO	0.9	NA	NA	92	117	45	2.60	78	56.4	11.1
2	35	M	NO	NO	NO	NO	NO	0.91	NA	NA	90	145	43	3.37	77	86.6	5.2
3	35	M	NO	NO	NO	NO	NO	0.92	NA	NA	81	155	39	3.97	67	102.6	11.6
4	30	M	NO	NO	NO	NO	NO	0.93	NA	NA	97	156	44	3.55	69	98.2	26
5	35	M	NO	NO	NO	NO	NO	0.92	NA	NA	88	158	28	5.64	129	104.2	14.3
6	32	M	NO	NO	NO	NO	NO	0.9	NA	NA	85	93	42	2.21	128	25.4	19.7
7	23	M	NO	NO	NO	NO	NO	0.91	NA	NA	143	169	43	3.93	52	115.6	10.2
8	30	M	NO	NO	NO	NO	NO	0.92	NA	NA	88	82	44	1.86	81	21.8	7.3
9	28	M	NO	NO	NO	NO	NO	0.93	NA	NA	100	205	42	4.88	66	149.8	17.2
10	39	M	NO	NO	NO	NO	NO	0.92	NA	NA	158	178	43	4.14	123	110.4	5.9
11	25	F	NO	NO	NO	NO	NO	0.85	NA	NA	78	137	43	3.19	79	78.2	18.3
12	35	M	NO	NO	NO	NO	NO	0.92	NA	NA	85	109	36	3.03	109	51.2	7.9
13	27	M	NO	NO	NO	NO	NO	0.9	NA	NA	77	102	38	2.68	134	37.2	12.1
14	40	M	NO	NO	NO	NO	NO	0.91	NA	NA	74	140	43	3.26	98	77.4	7.4
15	35	M	NO	NO	NO	NO	NO	0.92	NA	NA	80	108	52	2.08	123	31.4	8.4
16	29	M	NO	NO	NO	NO	NO	0.93	NA	NA	90	103	44	2.34	168	25.4	10.2
17	29	M	NO	NO	NO	NO	NO	0.96	NA	NA	59	88	38	2.32	123	25.4	14.2
18	31	F	NO	NO	NO	NO	NO	0.88	NA	NA	82	86	42	2.05	55	33	14.2
19	35	M	NO	NO	NO	NO	NO	0.9	NA	NA	103	123	31	3.97	120	68	19
20	38	M	NO	NO	NO	NO	YES	0.91	NA	NA	81	128	53	2.42	84	58.2	2.6
21	29	M	NO	NO	NO	NO	NO	0.92	NA	NA	86	110	54	2.04	119	32.2	5.9
22	33	M	NO	NO	NO	NO	NO	0.93	NA	NA	133	145	32	4.53	123	88.4	9.5
23	29	M	NO	NO	NO	NO	NO	0.92	NA	NA	103	131	48	2.73	154	52.2	59.7
24	32	M	NO	NO	NO	NO	NO	0.9	NA	NA	84	151	43	3.51	114	85.2	18.1
25	35	M	NO	NO	NO	NO	NO	0.91	NA	NA	79	103	50	2.06	129	27.2	4.5
26	35	M	NO	NO	NO	NO	NO	0.96	NA	NA	72	110	35	3.14	98	55.4	10.1
27	35	M	NO	NO	NO	NO	NO	0.97	NA	NA	88	149	34	4.38	124	90.2	11.1
28	35	F	NO	NO	NO	NO	NO	0.87	NA	NA	84	89	64	1.39	69	11.2	6.5
29	40	M	NO	NO	NO	NO	NO	0.93	NA	NA	78	89	43	2.07	79	30.2	18.3
30	28	M	NO	NO	NO	NO	NO	0.92	NA	NA	77	117	38	3.08	78	63.4	12.1

RESULTS

Table (1) : Characteristics of patients with CAD, their first degree relatives and controls

VARIABLES	CASES (30)	FIRST DEGREE RELATIVES (30)	CONTROLS (30)	P VALUE
AGE	38 ± 4.6	29 ± 14	28 ± 4.3	0.16 -NS
SEX M/F	26/4	23/7	27/3	0.33 - NS
DM	10	4	0	0.002 - S
HTN	7	0	0	0.001 –S
SMOKING	11	3	0	0.000- S
ALCOHOL	10	1	0	0.000 -S
WAIST HIP RATIO	0.95 ± 0.54	0.92 ± 0.04	0.91± 0.02	0.002 -S
FAMILY H/O PREMATURE CAD	12	-	1	0.001 -S
TOTAL CHOLESTEROL	171 ± 35.4	172 ± 44	125 ± 30.8	0.000 -S
HDL	31.3 ± 5.2	36.6 ± 5.3	42.4 ± 7.4	0.000 -S
TC:HDL RATIO	5.6 ± 1.4	4.7 ± 1.2	3.0 ± 1.0	0.000 -S
TRIGLYCERIDES	130 ± 74	125 ± 39	101 ± 30	0.078 -NS
LDL	113 ± 40.5	110 ± 37.9	63 ± 34.6	0.000 -S

S – significant, NS- not significant

Table (2) Family history of premature CAD in cases and controls

		CASECONT		Total
		CONTROLS	CASES	
FAMPCAD	NO	29	18	47
	YES	1	12	13
Total		30	30	60

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	11.882[a]	1	.001		
Continuity Correction[b]	9.820	1	.002		
Likelihood Ratio	13.569	1	.000		
Fisher's Exact Test				.001	.001
N of Valid Cases	60				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.50.
b. Computed only for a 2x2 table

Table (3) Distribution of Lp(a) levels between subjects

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Case	30	**37.0033**	**28.20837**	5.15012	26.4702	47.5365	5.90	89.10
First degree relatives	30	**26.9833**	**17.83540**	3.25628	20.3235	33.6432	5.60	76.40
Control	30	**13.2867**	**10.29763**	1.88008	9.4415	17.1319	2.60	59.70
Total	90	**25.7578**	**22.20451**	2.34056	21.1071	30.4084	2.60	89.10

ANOVA					
Lipoprotein(a)					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	8504.794	2	4252.397	10.458	**.000**
Within Groups	35375.786	87	406.618		
Total	43880.580	89			

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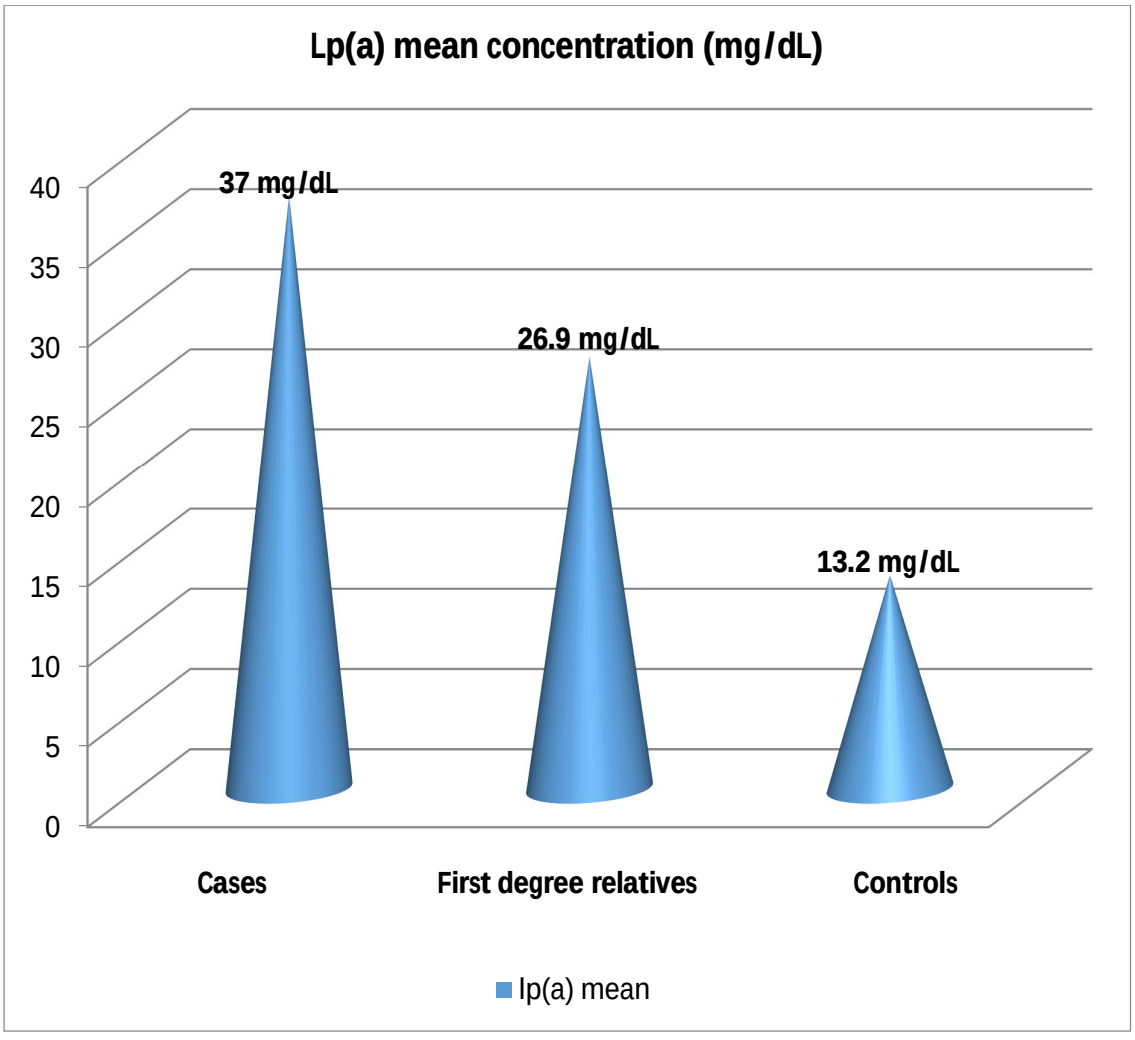
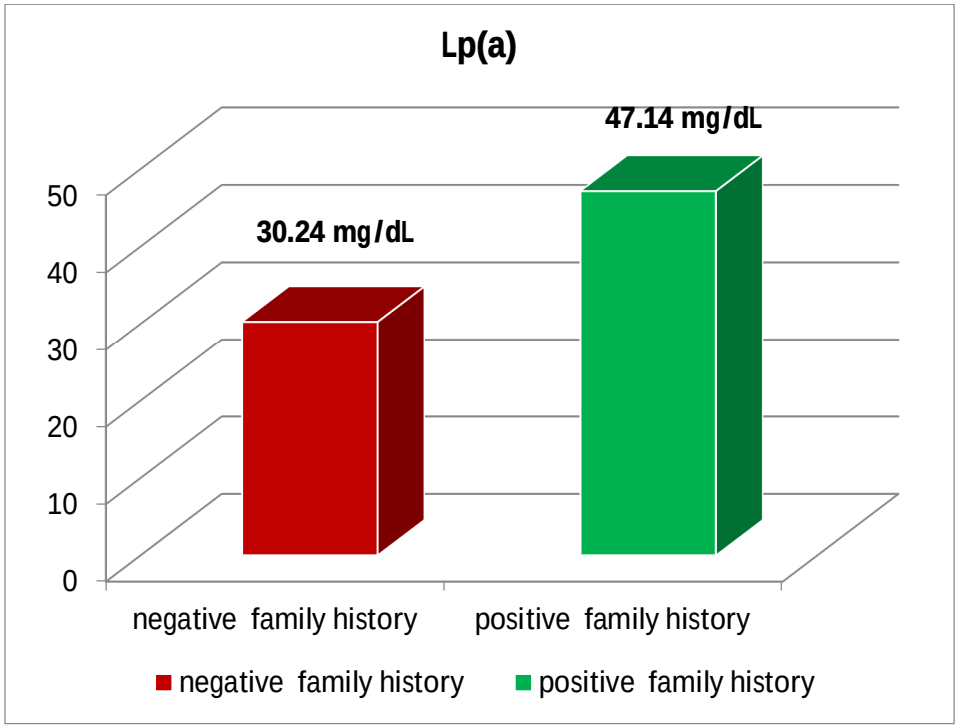
Fig 4. Mean plasma Lipoprotein(a) concentration in three groups



Table(4): Lp(a) concentration in patients with and without family H/O premature CAD

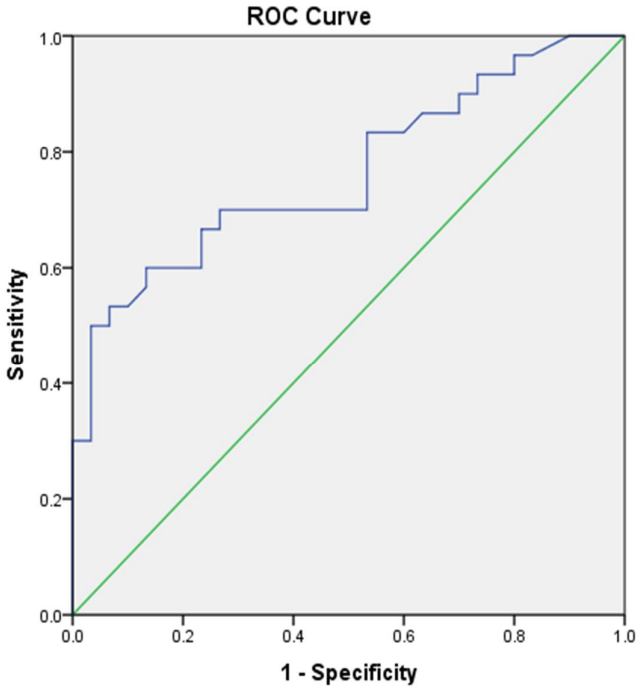
Family History of Premature CAD		N	Mean (mg/dL)	Std deviation	Std error of mean
Lp(a)	No history	18	30.244	27.23	6.41
	Pos history	12	47.14	27.65	7.98



RECEIVER OPERATING CHARACTERISTICS CURVE

Table (5) Demonstrated Cut off value for Lp(a) in our population

Positive if Greater Than or Equal To[a]	Sensitivity	1 - Specificity
1.6000	1.000	1.000
3.5500	1.000	.967
4.8500	1.000	.933
5.5500	1.000	.900
6.8000	.967	.800
7.5000	.933	.733
8.0500	.900	.700
8.9500	.867	.667
9.8000	.867	.633
10.1500	.833	.600
10.3000	.833	.533
10.9000	.767	.533
11.0500	.700	.533
11.3500	.700	.467
11.8500	.700	.433
13.1500	.700	.367
14.2500	.700	.300
14.9000	**.700**	**.267**
16.3500	.667	.267
17.4500	.667	.233
17.8500	.633	.233
18.0500	.600	.233
18.2000	.600	.200
18.3500	.600	.133
18.7000	.567	.133
19.3500	.533	.100
22.3000	.533	.067
25.4500	.500	.067
26.1000	.500	.033
27.9500	.467	.033
34.9000	.433	.033
42.6500	.400	.033
48.5000	.367	.033
55.3500	.333	.033
59.3000	.300	.033
60.4000	.300	.000
64.5000	.200	.000
69.3000	.167	.000
76.2000	.133	.000
80.8000	.100	.000



Area Under the Curve				
Test Result Variable(s): Lp(a)				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.763	.062	.000	.642	.885

Lp(a)	Cases(30)	First degree relatives(30)	Controls(30)
<30 mg/dL	17(56.7%)	19(63.3%)	29(96.7%)
>30 mg/dL	13(43.3%)	11(36.7%)	1(3.3%)
<14.9 mg/dL	9(30%)	11(36.7%)	22(73.3%)
>14.9 mg/dL	21(70%)	19(63.3%)	8(26.7%)

Table (6) Correlation between Lp(a) in patients and in their First degree relatives

Correlations			
		cases	First degree relatives
Cases	Pearson Correlation	1	.408*
	Sig. (2-tailed)		.025
	N	30	30
First degree relatives	Pearson Correlation	.408*	1
	Sig. (2-tailed)	.025	
	N	30	30
*. Correlation is significant at the 0.05 level (2-tailed).			

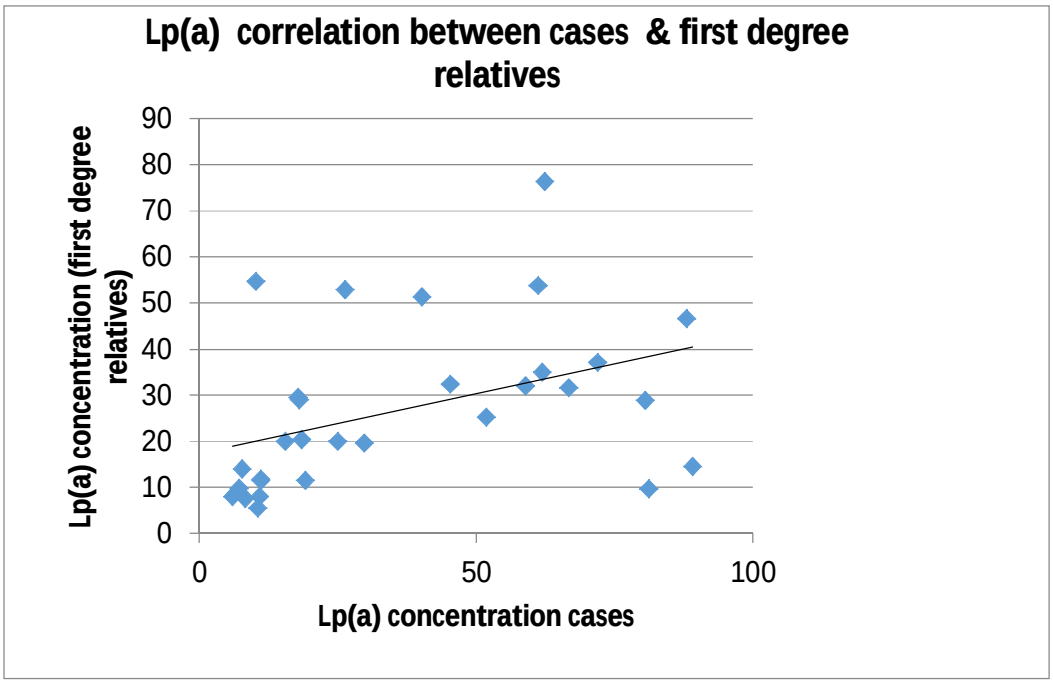
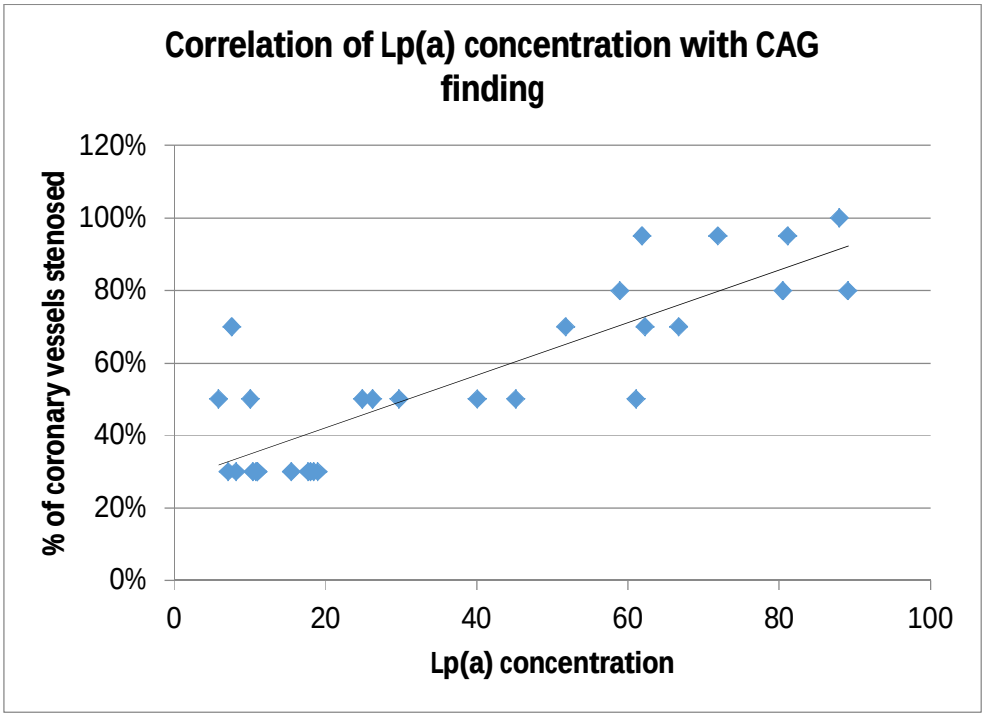


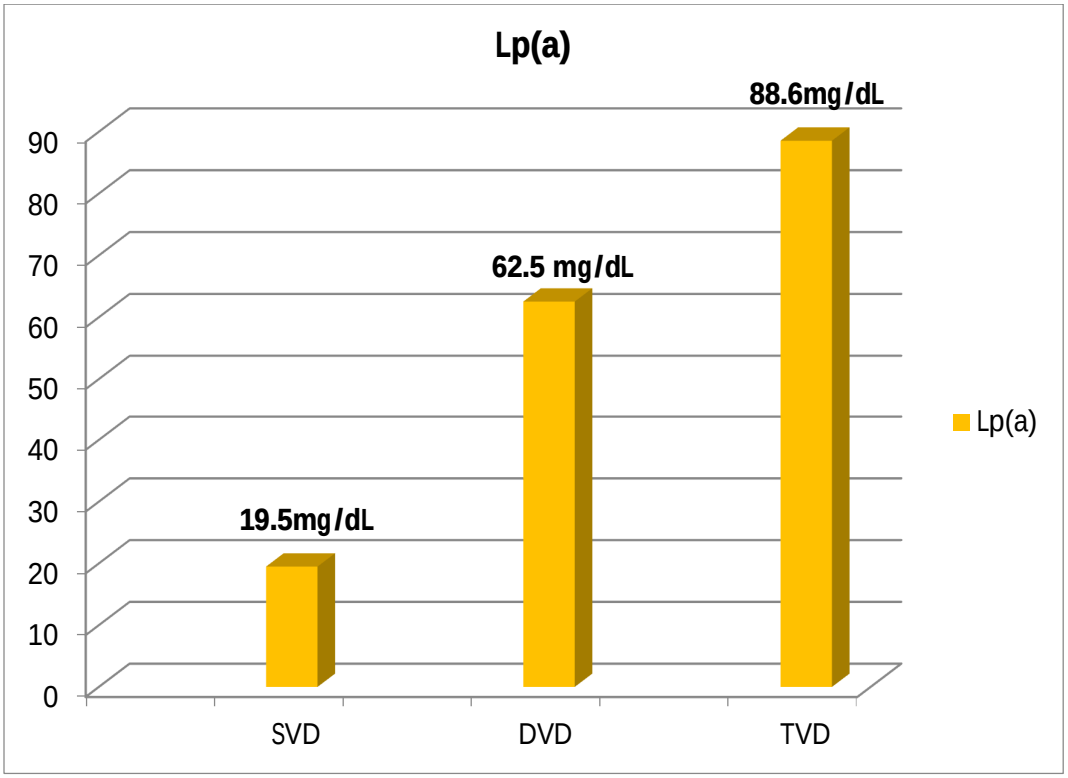
Table (7) Correlation between CAG Finding & Lp(a) in cases

Correlations			
		VAR00015	VAR00016
VAR00015	Pearson Correlation	1	.854**
	Sig. (2-tailed)		.000
	N	30	30
VAR00016	Pearson Correlation	.854**	1
	Sig. (2-tailed)	.000	
	N	30	30
**. Correlation is significant at the 0.01 level (2-tailed).			



Table(8) Lp(a) concentration and degree of vessel block

VESSEL BLOCK	MEAN Lp(a) mg/dL	STD DEVIATION	N
SVD	19.48	13.59	19
DVD	62.53	18.49	9
TVD	88.55	0.777	2



SVD- Single vessel disease

DVD- Double vessel disease

TVD- Triple vessel disease

Table (9) : Stepwise Regression Analysis

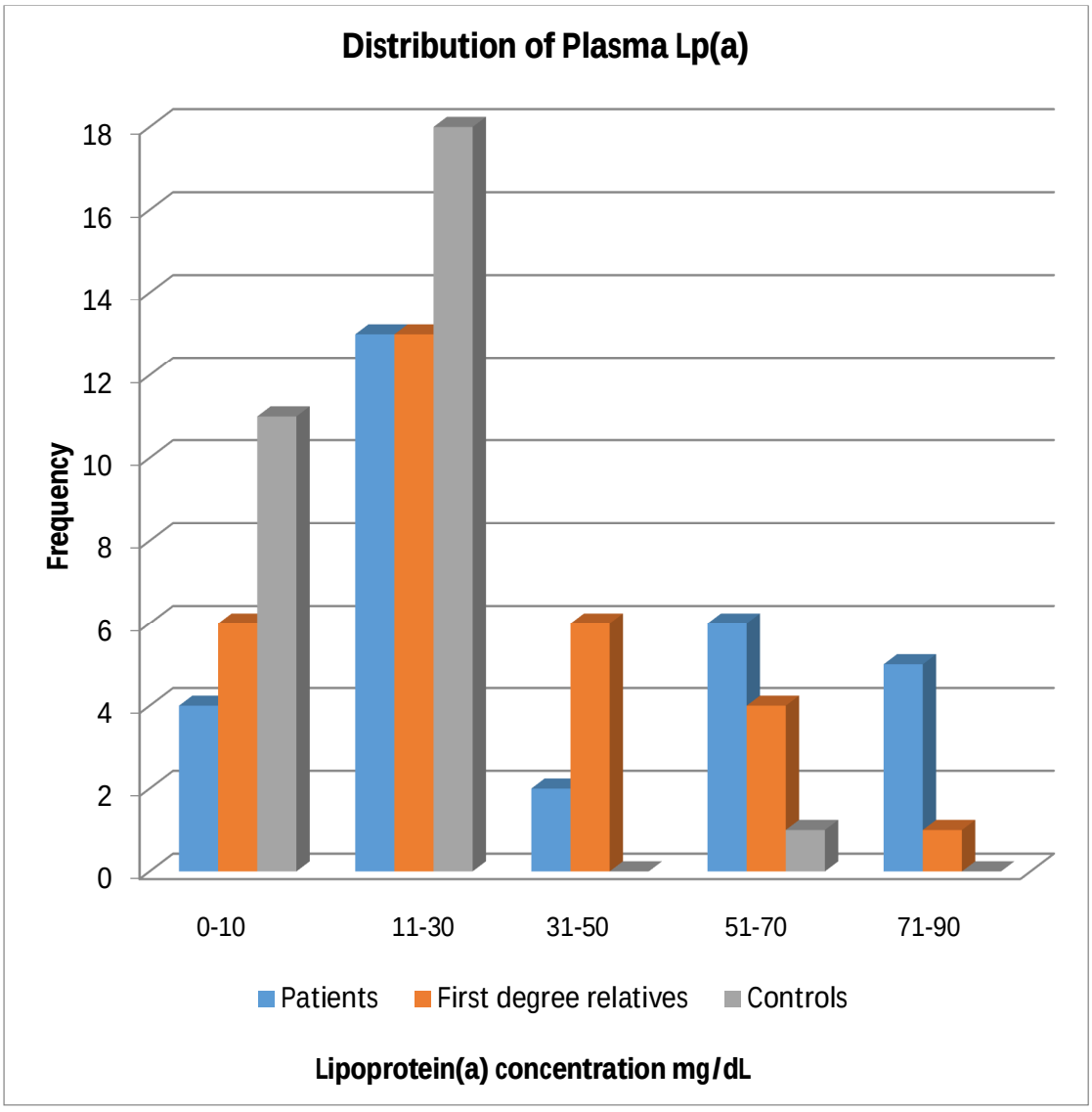
Model Summary[e]					
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.663[a]	.440	.430	.38067	
2	.739[b]	.546	.530	.34582	
3	.782[c]	.612	.591	.32252	
4	.817[d]	.667	.643	.30115	1.374

a. Predictors: (Constant), LDL
b. Predictors: (Constant), LDL, HTN1
c. Predictors: (Constant), LDL, HTN1, FPG
d. Predictors: (Constant), LDL, HTN1, FPG, LPA
e. Dependent Variable: CASECONT

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.595	.143		4.164	.000
	LDL	.010	.002	.663	6.746	.000
	(Constant)	-.191	.252		-.759	.451
2						
	LDL	.010	.001	.614	6.804	.000
	HTN1	.461	.126	.329	3.644	.001
	(Constant)	.102	.253		.402	.689
3						
	LDL	.009	.001	.576	6.772	.000
	HTN1	.472	.118	.337	4.000	.000
	FPG	-.002	.001	-.260	-3.088	.003
	(Constant)	.505	.271		1.863	.068
4						
	LDL	.008	.001	.506	6.123	.000
	HTN1	.382	.114	.273	3.346	.001
	FPG	-.002	.001	-.266	-3.388	.001
	LPA	-.005	.002	-.257	-3.038	.004

a. Dependent Variable: CASECONT

Fig 5.Frequency distribution of plasma Lp(a) levels among three groups



RESULTS

1. The mean age of cases was found to be 38±5, controls 28 ± 4 and first degree relatives 29 ± 14. The standard deviation for first degree relatives was high because the group included siblings and children covering a wide range of age. The first degree relatives included 16 siblings and 14 children of the 30 index patients under study.

2. Statistically there was no significant difference between sexes in all the three groups.

3. Table (1) shows there is a significant difference between groups with respect to Diabetes(p-0.002), Hypertension (p-0.001), smoking (p-0.000), alcoholism (p-0.000), waist hip ratio (p-0.002), family H/O premature CAD (p- 0.001), Total cholesterol (p-0.000), HDL (p-0.00), TC:HDL ratio (p-0.00), and LDL(p-0.00)

4. Total cholesterol was higher in patients(171±35) and their first degree relatives (172±44) compared to controls (125±30). In contrast HDL-C was higher in controls (42±7) compared to patients (31±5) and first degree relatives (36±5). Triglycerides and LDL were higher in patients and their relatives than controls.

5. Table (2) shows 12 patients had family history of premature CAD whereas only 1 had family history in controls.

6. Table (3) shows the mean Lp(a) concentration observed in cases 37±28 mg/dL and in their first degree relatives it is 27 ± 18 mg/dL. In controls it was found to be less 13±10 mg/dL compared to cases. This indicates that the genetic predisposition of Lp(a) makes the relatives at risk population for future CAD. P value observed between groups was very significant- 0.000.

7. Figure 5 shows the frequency distribution of plasma Lp(a) concentration in three groups. Control group showed higher frequency when lipoprotein(a) was <30 mg/dL. As the concentration increased more than 30 mg/dL cases and their first degree relatives showed higher frequency compared to controls.

8. The mean Lp(a) concentration in patients with positive family history of premature CAD was 47.1 mg/dL and without positive family history is 30.2mg/dL. (Table 4)

9. Table (5) shows the true positive and false positive rates for different levels of Lp(a). True positive is given by sensitivity and false positive is mentioned as 1-specificity. This table was drawn to distinguish cases and controls. The cut off value for Lp(a) above which there are high true positives and less false positives was noted and found to be 14.9 mg/dL (70% sensitivity and 73% specificity). Therefore the cut off value already in use which is 30 mg/dL may not be applied in our population and there is a chance to miss some of the cases at risk.

10. Receiver Operating Characteristics curve is drawn with true positives against false positives. Area under the curve obtained is 0.763, standard error (under the non parametric assumption) is 0.062 and 95% confidence interval is 0.642 to 0.885.

11. Table (6) shows there is significant positive correlation between Lp(a) levels in patients with the Lp(a) levels of their first degree relatives as expected. Out of 30 families evaluated-16 were siblings and 14 were children of the index patients.18 patients and one of their first degree relatives had Lp(a) > 14.9 mg/dL . 11 patients and one of their first degree relatives had Lp(a) > 30 mg/dL. This signifies the genetic predisposition of Lp(a) in families which stays to be the contributing factor for CAD risk in them in future.

12. Tables (7 and 8) show the correlation between between Lp(a) concentration and the severity of block noticed in coronary angiogram in patients. Significant correlation was obtained between the levels and the severity of lesion as well as number of vessels involved i.e., atherogenicity of Lipoprotein(a) increases with its increase in concentration.

13. Table(9) shows the stepwise regression analysis to find out the independent predictors for CAD. Independent variables entered into the analysis were Diabetes mellitus, Hypertension, smoking, alcoholism, Waist hip ratio, fasting plasma glucose, LDL-C, HDL-C, triglycerides and Lp(a) with CAD as dependant variable. Out of these, LDL-C, Hypertension, fasting plasma glucose and Lp(a) were found to be the independent predictors of CAD risk.

Discussion

DISCUSSION

Coronary artery disease (CAD) is the principal cause of mortality and morbidity in the developed countries. However recent evidences show that there is an alarming increase in the prevalence of coronary artery disease in South Asians. In particular, there is an upsurge in the incidence of premature CAD in the young population in the recent decades. The mortality and morbidity from CAD impairs the economic productivity of the person in his lifetime. Several causes for premature CAD as identified in WHO based MONICA study,[125] Euroheart ACS epidemiological studies [126] are smoking, family history of premature CAD, familial hypercholesterolemia, homocystinemia, obesity, physical inactivity, depression and mental stress.

Goel et al,[127] in their study found that the family history of premature CAD became the second most important risk factor in young Indian CAD patients. Therefore analysis of genetic factors that plays a role in atherogenesis , thrombogenesis, thrombolysis , lipid metabolism, and other metabolic factors is needed. Lp(a) remains to be one of the potential culprits for the development of premature CAD in Indians. A strong genetic predisposition of Lp(a) holds the upcoming generation of the affected population at risk for developing CAD in future. Limited studies on Lp(a) have been done in our Indian population because of the challenges in its measurement and currently no effective treatment that brings down its level specifically has been identified.

The present study emphasises the association of Lipoprotein(a) levels in angiographically proven young CAD patients less than 45 years of age with their first degree relatives. Lp(a) levels has shown wide variation between different ethnic population and its propensity to cause CAD also varies between races. African population and Indians both have higher Lp(a) concentration yet the incidence of CAD is less in Africans compared to Indians.[128] This may be explained because of the prevalence of atherogenic isoforms or the specific single nucleotide polymorphism in LPA gene which enhances the risk for CAD among Indians.

The study was designed to determine the following .

1. Whether there is increased Lp(a) levels in young CAD patients who survived the incidence of premature CAD?
2. Whether there is associated increase in Lp(a) levels in the first degree relative of the patients?
3. Whether Lp(a) can be included along with other risk assessment parameters for CAD in our population?
4. To determine the cut off value for our population.

Male gender is one of the non modifiable risk factors for CAD. As is the case, male preponderance was observed in our study. The non significant P values obtained for age and sex denotes that both are matched between cases and controls. Statistically significant difference exists between the three groups with respect to the conventional risk factors like Diabetes mellitus, Hypertension, smoking, alcoholism, abdominal obesity (measured in terms of waist hip ratio)

which may be an added contribution to the CAD incidence in the cases. Positive family history of premature CAD strongly determines the risk for premature CAD.[129] Here significant number of cases documented the family history of premature CAD compared to control group (p value-0.001).

Inspite of patients being on statin therapy and the lipid parameters were within normal limits, the mean total cholesterol of 171 mg/dL and LDL cholesterol of 113 mg/dL were on the higher side compared to the control groups with mean total cholesterol 125 mg/dL and LDL cholesterol 63 mg/dL respectively. The inefficacy of statins to bring down the higher Lp(a) concentration observed in these individuals is questioned. As expected lower HDL concentration of 36 mg/dL was observed in cases (p value -0.000).

Ghambir et al in the epidemiological studies conducted in India have documented that Lp(a) is an independent risk factor for CAD in patients below 40 years of age. [130,131] Similarly significant difference in Lp(a) concentration was observed between three groups, with cases having the highest mean Lp(a) concentration(37 ± 28 mg/dL), control group having the least (13 ± 10 mg/dL) and first degree relatives having levels intermediate between cases and controls (26 ± 17 mg/dL). (p value- 0.000)

As mentioned previously, studies done in Caucasian population established 30 mg /dL as the cut off value for risk assessment but data from recent multi ethnic study[132] reveals 30 mg/dL can be used for Black population, whereas 50 mg/dL which is higher than the previous cutoff should be considered appropriate for Caucasian and Hispanics. Because of this diversity in the reference range in

Fig 6. Distribution of Lp(a) among cases

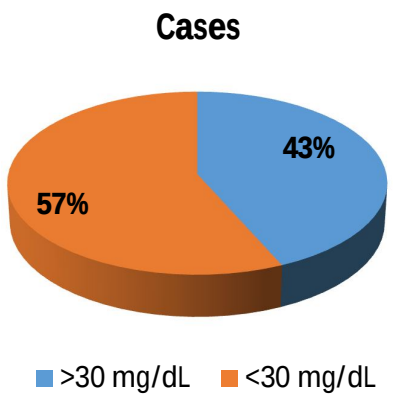
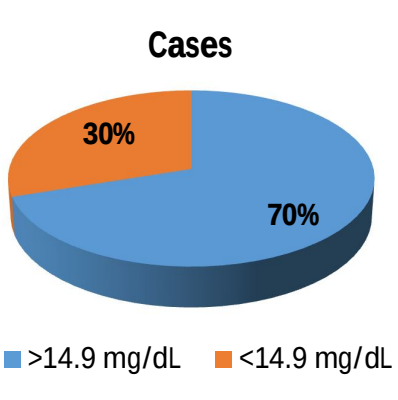
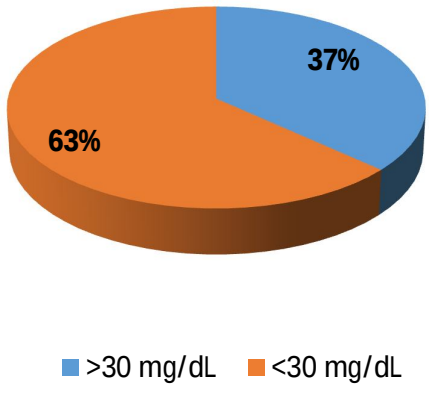
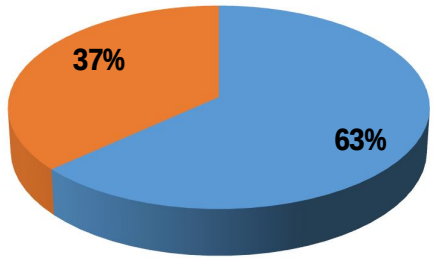
i) At cut off point 30 mg/dL	ii) At cut off point 14.9 mg/dL
	

Fig 7. Distribution of Lp(a) among first degree relatives

i) At cut off point 30 mg/dL	ii) At cut off point 14.9 mg/dL
	

each population, race specific cut off value should be determined for Indian population with clinical correlation. The cut off value in our study derived with 70% sensitivity and 73% specificity is 14.9 mg/dL which means the current guidelines may mask some of the patients at risk.

Prevention of coronary heart disease starts with screening of individuals with overall high risk. The first degree relatives involving the siblings and offsprings of the patients with premature CAD are the potential targets for intervention, since the family members share a common lifestyle and genetic predisposition. In EUROASPIRE II (European Action on Secondary Prevention by Intervention to Reduce Events) family survey[133,] 3322 first degree relatives (siblings and children > 18 years of age) of 1289 index patients who survived the incidence of premature CHD (men under 55 years and women under 65 years) were questioned whether screening for coronary risk factors had been done and, if so, whether lifestyle modifications and drug therapies were given to them. It was found that only 11% of siblings and 5.6% of children were screened. In our study, one of the genetic risk factors for CAD viz., lipoprotein(a) was screened among the first degree relatives (siblings and children irrespective of age). We found that there was a positive correlation for the presence of risk factor among the first degree relatives and the percentage of which varied depending on the cut off value of Lp(a).

- When we considered 30 mg/dL as the cut off point , 37% of the first degree relatives of the index patients had risk for CAD.
- However when the cut off value of 14.9 mg/dL was considered we found that 60% of the first degree relatives of the index patients had risk for CAD.

Coronary angiogram findings of the patients i.e the severity of block and the number of vessels affected have been correlated with Lp(a) levels. Different Lp(a) concentration have been observed with differing grades of diseased vessels. In the study done by Fauzia Ashfaq et al, among North Indian patients[134] Lp(a) concentration with normal coronaries was found to be 18.9 mg/dL, patients with Grade 1 vessel had Lp(a) of 39.2 mg/dL, Grade 2 vessel with 58 mg/dL and Grade 3 vessel with 69.2 mg/dL. Similarly we have also observed a positive correlation of lipoprotein(a) levels with the severity of blockage as well as the number of vessels involved (SVD,DVD,TVD).In patients with single vessel block, the mean Lp(a) level was 19.4 mg/dL and in Double Vessel Disease the mean Lp(a) was 62.5 mg/dL , while in patients with Triple Vessel Disease,the mean value was 88.5 mg/dL. This clearly shows that elevated Lp(a) adds to the severity of atherosclerotic changes and involves multiple vessels. Correlation was significant at 0.01 level . In a study by Ghambir et al, assessment of independent predictors of CAD risk was carried out in a multivariate analysis out of which smoking, Lp(a), HDL-C and Triglycerides entered the model. In the present study, LDL-C, Hypertension, Fasting plasma Glucose and Lipoprotein(a) were found to be the independent predictors.[135]

Conclusion

CONCLUSION

1. In our study we have analysed three groups -angiographically proven young coronary artery disease patients aged <45 years, their first degree relatives and age and sex matched healthy controls.
2. We found that Lp(a) was elevated in the young CAD patients and there was associated elevation in their first degree relatives. 18 patients and their first degree relatives (out of 30 families evaluated) had elevated Lp(a). Lp(a) levels also correlated with the number of vessels affected and the severity of block .
3. Unexpectedly, our population had a lower cut off value for Lp(a) of 14.9 mg/dL, which means our population have a higher risk for developing CAD at lower levels of Lp(a) compared to other population and the people whose Lp(a) levels fall between 15-30 mg/dL cannot be overlooked.
4. In our study Lipoprotein (a) is found to be the independent predictor for coronary artery disease along with high LDL, Hypertension and elevated fasting plasma glucose.
5. Since this genetic factor remains as a silent potential culprit for developing CAD, Lp(a) has to be included as one of the lipid profile parameters of the patients attending hospital and in health screening in our population.
6. The study shows a strong genetic predisposition , thus the Lp(a) screening at relatively younger age for the first degree relatives of patients with premature CAD has to be encouraged. Hence primary prevention of CHD can be achieved by providing cost effective measures before the disease is being well established.

Limitation of the study

LIMITATIONS OF THE STUDY

1. The study didn't exclude conventional risk factors like Diabetes, Hypertension and smoking which are the additional risk factors for developing the disease.
2. Controls were not subjected to Treadmill test or angiography for exclusion because of ethical reasons.
3. Only one of the first degree relatives of the patient was screened for Lp(a) elevation. Whole family could not be screened due to the poor compliance and the cost of the analysis.
4. We could not strictly adhere to the guidelines for measurement of lipoprotein(a) as mentioned earlier(5)
    - Lp(a) levels was measured in terms of mass i.e. in mg/dL instead of its expression in nmol/L.
    - Calibrator used in the Lp(a) assay was traceable to WHO Reference material SRM2B . However the sensitivity of the method used to measure Lp(a) with respect to isoform size could not be ruled out.

Scope for further studies

SCOPE FOR FURTHER STUDIES

1. Population and ethnicity based reference interval for Lp(a) has to be determined.
2. The physiological and pathological role of Lp(a) still needs to be established
3. Whether determining isoform size in an individual would be a better indicator than Lp(a) for CAD risk has to be clarified.
4. Single nucleotide polymorphisms affecting Lp(a) concentration at loci other than *LPA* loci have to be identified. (like apo E gene, IL-6 gene already in study)
5. Drugs that specifically targets Lp(a) should come up, so that therapeutic measures can be implemented.

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Annexures

PROFORMA

Name: Age/Sex:

Address: IP/OP No:

Ph no:

Occupation:

RELEVANT HISTORY:

Family H/O premature CAD H/O similar illness in the past:

K/C/O: HTN/DM/IHD/Stroke

Smoking: Regular exercise:

Diet: veg/ non veg

Drug History:

GENERAL EXAMINATION :

BMI: Waist Hip ratio:

SYSTEMIC EXAMINATION:

CVS: RS: P/A: CNS:

VITAL PARAMETERS:

Pulse: BP:

DIAGNOSIS:

INVESTIGATIONS:

Coronary Angiography finding:

Lipid profile: TC: HDL-C: TC-HDL-C ratio:

Triglycerides: LDL-C:

Lp(a) :

INFORMATION SHEET

- Your blood sample has been accepted.
- We are conducting a study on patients with Coronary artery disease at Rajiv Gandhi Government General Hospital, Chennai and for that your blood sample may be valuable to us.
- The purpose of this study is to correlate the level of lipoprotein(a) and apo(a) isoform size in young coronary artery disease and their first degree relatives with the help of certain special tests.
- We are selecting certain cases and if your blood sample is found eligible, we may be using your blood sample to perform extra tests and special studies which in any way do not affect your final report or management.
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of investigator Signature of participant

Date:

PATIENT CONSENT FORM

Title of the study **: A STUDY ON LIPOPROTEIN (a) LEVELS AND ITS CORRELATION WITH APO(a) ISOFORM SIZE IN YOUNG CORONARY ARTERY DISEASE PATIENTS AND THEIR FIRST DEGREE RELATIVES.**

Name :
Age :
Sex :

Date :
OP No :
Project Patient No :

The details of the study have been provided to me in writing and explained to me in my own language.

I confirm that I have understood the above study and had the opportunity to ask questions.

I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected.

I agree to use my personal clinical history& investigation details for the purpose of the study.

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

I have been given an information sheet giving details of the study.

Having understood _____s/o_____ give my consent to participate in the study conducted by DR.A.K.ROOPA, Post graduate, Institute of Biochemistry, Madras Medical College, Chennai.

Signature of the investigator:
Place:
Date:

Signature of the participant:
Thumb impression.

நோயாளியின் ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு: **CµzuUSÇõ´ \õº¢u C¸u¯ ÷{õ¯õ Î PÐUS®, AÁ¸øh¯ ¬uÀuµ EÓÂÚºPÐUS® öPõÊ¨¦ ¦µu®&a °ß AÍÄ AuÝhß A¨÷£õöPõÊ¨¦ ¦µu®&a °ß C÷\õ£õºªß AÍøÁCø n¯ÓÄ ö\´²®J¸©¸zxÁ B´Ä.**

பெயர் : தேதி :

வயது : புறநோயாளிஎண்:

பால் : ஆராய்ச்சி சேர்க்கை எண் :

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது.

எனக்கு விளக்கப்பட்ட விஷயங்களை புரிந்து கொண்டு நான் எனது சம்மதத்தைத் தெரிவிக்கிறேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில் தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும், அதனால் எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்து கொண்டேன்நான் "**CµzuUSÇõ´ \õº¢u C¸u¯ ÷{õ¯õ Î PÐUS®, AÁ¸øh¯ ¬uÀuµ EÓÂÚºPÐUS® öPõÊ¨¦ ¦µu®&a °ß AÍÄ AuÝhß A¨÷£õöPõÊ¨¦ ¦µu®&a °ß C÷\õ£õºªß AÍøÁCø n¯ÓÄ ö\´²®J¸©¸zxÁ B´Ä**" என்ற தலைப்பில் மேற்கொள்ளப்படும் இந்த ஆராய்ச்சியின் விபரங்களைக் கொண்ட தகவல் தாளைப் பெற்றுக் கொண்டேன்.

இதன் மூலம் எந்த பின்விளைவும் வராது என மருத்துவர் மூலம் தெரிந்து கொண்டு என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக்கொள்ள சம்மதிக்கிறேன்

தேதி கையொப்பம

ஆராய்ச்சி தகவல் தாள்.

தங்களது இரத்தம் இங்குபெற்றுக்கொள்ளப்பட்டது.

சென்னை அரசுபொது மருத்துவமனையில் "CµzuUSÇõ´ \õº¢u C¸u¯ ÷{õ¯õ Î PÐUS®, AÁ¸øh¯ ¬uÀuµ EÓÂÚºPÐUS® öPõÊ¨¦ ¦µu®&a °ß AÍÄ AuÝhß A¨÷£õ öPõÊ¨¦ ¦µu®&a °ß C÷\õ£õºªß AÍøÁ Cø n¯ÓÄ ö\´²® J¸©¸zxÁ B´Ä" என்ற தலைப்பில் ஆராய்ச்சி நடைபெற்று வருகின்றது.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்பிகிறோம். இந்த ஆராய்ச்சியில் உங்களுடைய இரத்தம் எடுத்து சிறப்புப்பரி சோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வறிக்கையோ அல்லது சிகிச்சையோ பாதிப்புக்கு உள்ளாகாது என்பதையும் தெரிவித்துக்கொள்கிறோம்.

முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்ச்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்துக்கொள்கிறோம்

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது.மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துகொள்கிறோம்.

இந்த சிறப்பு பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போது அல்லது ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

தேதி:

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr. A.K.Roopa,
II nd Year, PG Degree M.D BioChemistry
Institute of Biochemistry
Madras Medical College, Chennai

Dear ,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY ON LIPOPROTEIN (A) LEVELS AND ITS CORRELATION WITH APO(A) ISOFORM SIZE IN YOUNG CORONARY ARTERY DISEASE PATIENTS AND THEIR FIRST DEGREE RELATIVES " NO.06092016 .**

The following members of Ethics Committee were present in the meeting hold on **06.09.2016** conducted at Madras Medical College, Chennai 3

1. Prof. C. Rajendran, MD.	Chairperson
2. Prof. Dr. M.K. Muralidharan, M.S, M.Ch., MMC ,Ch-3	Deputy Chairperson
3. Prof. Sudha Seshayyan, MD., Vice Principal, MMC.Ch- 3.	Member Secretary
4. Prof. B.Vasanthi,MD.,Prof of Pharmacology, MMC,	Member
5. Prof. P.Raghumani.MS., Professor of Surgery, Inst. of surgery	Member
6. Prof. R.Padmavathy,MD., Professor, Inst.of Pathology, MMC,Ch	Member
7. Tmt.J.Rajalakshmi, Junior Administrative Officer,MMC,Ch	Layperson
8. Thiru.S.Govindasamy., B.A.B.L., High Court, Chennai-1	Lawyer
9. Tmt.ArnoldSaulina, MA., MSW.,	Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

MADRAS MEDICAL COLLEGE * CHENNAI-600 003 * 21 SEP 2016

25/9

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE.
MADRAS MEDICAL COLLEGE
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PLAGIARISM CERIFICATE

This is to certify that this dissertation work titled **"A STUDY ON LIPOPROTEIN(a) LEVELS IN YOUNG CORONARY ARTERY DISEASE PATIENTS AND THEIR FIRST DEGREE RELATIVES"** of the candidate **DR.A.K.ROOPA** with registration Number **201523004** for the award of **M.D** in the branch of **BIOCHEMISTRY**. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **7 percentage** of plagiarism in the dissertation.

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